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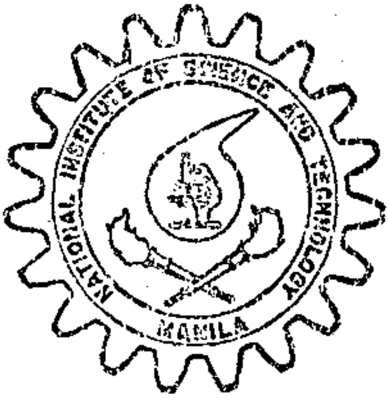
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THE NATA ORGANISM — CULTURAL REQUIREMENTS, CHARACTERISTICS, AND IDENTITY

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(Received for publication, April 21, 1967.)

TWO PLATES

INTRODUCTION

A delicacy among Filipinos, popularly known as "nata" is well-liked for dessert. It is a white or cream colored cartilaginous substance which is formed by a species of an acetic acid-forming bacterium on the surface of saccharine solutions enriched with fruit juices or any other nutritive plant extract. It is sold in the market as either *nata de piña* or *nata de coco,* etc., trade designations derived from the names of the fruits which are used to enrich the medium in which the nata organism is cultivated.

The nata organism is widely distributed in nature. It may be obtained from fermenting sweet plant juices or rotting sugary fruits and vegetables. Its isolation from any of these materials is not difficult to perform provided that it is grown on agar medium which has been sweetened with cane sugar and enriched with fruit juice or yeast extract.

Nata-making may play a role of importance in the development of the coconut industry in the Philippines because of the

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growing interest in its production from coconut water, an abundant waste product of copra manufacture.

In 1964 the Philippines produced 7,222,164,200 nuts valued at 661,454,500 pesos.[2] Since there is no report on how the water from those nuts was disposed of, it is presumed that much of it was wasted, particularly in regions where the copra was manufactured. Of the above nut production 6,508,229,900 were used for copra manufacture from which approximately 1,301,645,980 liters[3] of coconut water could be obtained. If half of this volume which amounts to 650,822,990 liters, was used as culture medium for nata organism, about 195,246,897 kilos of raw nata[4] could be produced. At a minimum price of 30 centavos a kilo of the raw product about 49,572,069 pesos could be realized from culturing nata organism in coconut water.

Nata-growing in coconut water in this country is still dependent on chance or on the skill and experience of the culturists, some of whom do not know that nata is formed by a living bacterium, which, like many other minute organisms, is sensitive to variations in the physical or chemical nature of the surroundings. The failure of many nata culturists to produce massive formation of the desired substance may be due to lack of knowledge of the behavior of the organism, inadequacy of information on factors conducive to its best development and nonobservance of sanitary and other precautionary measures for ridding the cultures of the inroads of pernicious and undesirable contaminants.

REVIEW OF LITERATURE

There exists some confusion regarding the true identity of the nata organism. This conflict might have arisen from inadequate knowledge of pertinent characteristics of the organism and from inaccuracy of observations on relevant facts about its behavior and activities.

In his study on the production of "nata de piña" from sugared pineapple pulp mash Villanueva,(20) one of the earliest investigators to report on this product believed to be chiefly dextran, hinted that its causal agent is a bacterium belonging to genus *Leu-*

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[2] Compiled from records of the Statistical Division, National Economic Council, U. P. Alumni Building, Padre Faura, Manila.

[3] Computed on the basis of an average of 200 cc or 0.2 liter per nut.

[4] Based on a yield of 0.3 kilo of raw nata per liter of coconut water.

conostoc. The nata-forming organism was first referred to as *Leuconostoc mesenteroides* (Cienkowski) van Tieghem by Adriano, et al.(1) who described the product that it forms in pineapple juice as a mucilaginous substance which consists of dextran. Apparently, following similar trend, Africa,(2) who studied the production of nata from coconut water included in her report a review of the history of dextran-forming *L. mesenteroides* for the nata organism which is considered to be identical with the one under study. Mendoza(13) named the nata-forming organism *Acetobacter aceti* (Kutzing) Beijerinck and described it as a capsulated bacterium which "produces a gelatinous substance on the surface of a suitable substrate of proper acidity and sugar solution." He made no detailed description of the organism to support and validate the correctness of its taxonomic position in the bacteriological realm. Apparently, he might have observed that it is a bacillus and that, nata, one of the main products of its activity is usually associated with acetic acid formation in the presence of tolerable amount of alcohol and sucrose undergoing a process of inversion followed by alcoholic fermentation. In his study of the optimum conditions for "nata de coco" bacterium, Alaban(3) included in his report a discussion of its taxonomy pointing to its similarity to the dextran-forming *L. mesenteroides*. The above conflict could only be resolved when the consensus confers only one binomial for the organism found constantly associated with nata formation in saccharine solutions.

PURPOSE OF THE INVESTIGATION

The purpose of the present investigation is to gather more information on how the nata organism could be induced to produce impressively high yields of nata and to study its essential morphological and cultural features and physiological characteristics which may lead to a clarification of its conflicting identity.

MATERIALS AND METHODS

The nata organisms were isolated from rotting fruits of mango (*Mangifera indica* Linn.), guava (*Psidium guajava* Linn.), pineapple [*Ananas comosus* (Linn.) Merr.], sineguelas (*Spondias purpurea* Linn.), chico (*Achras sapota* Linn.), and ates (*Anona squamosa* Linn.) and from fermenting vinegar which showed a cartilaginous growth on its surface. The presence of the organism in a rotting fruit was determined by allowing it to grow and produce the cartilaginous substance in a mixture of mashed tissues from lightly infected portions of the fruit and sterilized 5-per cent sugar solution in water or tomato-peptone-yeast-sucrose medium in a paper-covered sterilized glass jar. The organism causing the cartilaginous formation was isolated by the usual pour-plate-dilution method on tomato-peptone-yeast-sucrose agar. A more or less similar method was followed in isolating the same organism from fermenting vinegar which shows formation of the cartilaginous substance.

All of the isolates were studied comparatively for thickness of nata formed in tomato-peptone-yeast-sucrose solution. The isolate which produced the thickest formation of nata was selected and used in succeeding studies discussed here.

The experiments on cultural requirements and environmental factors for optimum development of nata organism which were conducted in the laboratory under aseptic conditions had to be centered to the relation of pH to nata formation using a nutrient medium[5] and a coconut-water medium[6] previously found to favor nata formation; the influence of temperature on nata formation in a synthetic medium of similar composition; the determination of the kind of sugar which would favor thick formation of nata in a basal medium[7] shown below; the selection of cheap and

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[5] Formula of liquid medium used in the study on the effect of pH on nata formation:

Potassium phosphate, monobasic (KH_2PO_4)	0.4 gm
Magnesium sulfate ($MgSO_4.7H_2O$)	0.4 gm
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 gm
Succinic acid $(CH_2COOH)_2$	1.0 gm
Yeast extract (Bacto)	2.5 gm
Cane sugar ($C_{12}H_{22}O_{11}$)	50.0 gm
Water to make	1000.0 cc

[6] Coconut-water medium:

Coconut water	1000.0 ml
Cane sugar	50.0 gm

[7] Basal medium:

Yeast extract	2.5 gm
Peptone	5.0 gm
Potassium phosphate, monobasic (KH_2PO_4)	1.0 gm
Magnesium sulfate ($MgSO_4.7H_2O$)	0.5 gm
Sodium chloride (NaCl)	1.5 gm
Water to make	1000.0 cc
pH	5.0

readily available form of nitrogenous substances which could promote thick formation of nata. Experiments covering the above subjects were carried in 8- by 1-inch culture tubes and in 500-cc clean dextrose bottles containing, respectively, 20 cc and 100 cc of the media. After sterilizing at 15 pounds pressure for 20 minutes they were cooled down to room temperature. They were then inoculated each with a young culture of nata organism and then incubated at room temperature (28°–31°C) for 15 days. The nata formed in each tube was gathered, dehydrated by dipping in 95-per cent ethyl alcohol and then measured by water displacement in a glass cylinder graduated to 1-cc units.

After determining that $NH_4H_2PO_4$ was the best source of nitrogen for nata organism its stimulative effect on nata formation was further tested by growing the organism in different concentrations of this nitrogenous compound in sugar coconut skim milk medium.[8] The experiment was performed in two series with practically no difference in detail except the method of heating the medium to eliminate microbial contaminants. In the first series the sugared coconut skim milk medium was autoclaved for 20 minutes at 15 pounds pressure while in the second series it was heated to boiling point and kept at boiling temperature for 5 minutes. After cooling, the medium in each case was distributed in volumes of 400 cc into 1-liter jars which were then divided into different batches by adding $NH_4H_2PO_4$ computed to be present in concentrations of 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, and 1.3 per cent. A batch without $NH_4H_3PO_4$ was kept as control. Each batch was adjusted to pH 5 and then inoculated with 5 cc of a 48-hour-old culture of the nata organism in coconut water medium. After incubating the cultures for 15 days at room temperature the nata formed from each batch was harvested, surface-dehydrated in 95-per cent ethyl alcohol and then weighed.

The identity of the selected nata organism was determined by studying its morphological and cultural characteristics and bio-

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[8] Preparation of coconut skim milk medium:

Grated coconut meat from mature nuts	1 kilo
Water	4 liters

Mix a little water with the grated coconut meat. Squeeze thoroughly the mixture to extract the milky substance or "gata." Add the rest of the water, stir, and then strain the "gata" through cheesecloth into a percolator. Allow the milky liquid to stand for sometime until the oily cream has all floated. Drain the lower layer and add to it white cane sugar at the rate of 100 grams per liter.

chemical reactions and comparing them with those described in Bergey's Manual of Determinative Bacteriology.(6)

The presence of cellulose in nata was tested by Miller's bromine process described by Hess, et al.(10) as follows:

> A 2-gram sample of nata in 10-per cent potassium hydroxide solution was boiled for about 20 minutes and then washed with bromine water. The resulting substance was dried in the oven at 60°C and then tested for cellulose by the sulfuric acid-iodine treatment using cotton for comparison. A blue color which indicated the presence of cellulose was verified by the Sweitzer's Reagent Test described by Leach(11) in his book titled "Food Inspection and Analysis."

RESULTS AND DISCUSSION

CULTURAL REQUIREMENTS OF THE NATA ORGANISM

Relation of pH to nata formation.—The results shown in Table 1 indicate that the nata organism is distinctly acid-tolerant thriving even as low as pH 3.5. With pH 7.5 as the maximum critical limit of tolerance, it is seen that the range of hydrogen-ion

TABLE 1.—*Effect of pH on nata formation and yield.*

	Nutrient medium		Coconut water medium	
Initial pH	Observation on nata formation	Average yield after 15 days incubation	Observation on nata formation	Average yield* after 15 days incubation
		Cm^3		*Cm*^{3}
2.0	No nata formation	none	No nata formation	none
2.5	do	none	do	none
3.0	do	none	do	none
3.5	Thin, soft	3.5	Thin, soft	4.5
4.0	Moderate, soft	5.0	Moderate, firm	7.16
4.5	Thick, firm	9.27	Thick, firm	9.16
5.0	do	6.9	do	12.00
5.5	Moderate, firm	8.17	do	12.00
6.0	do	8.17	do	9.60
6.5	Moderate, soft	6.27	Moderate, firm	8.00
7.0	Thin, soft	4.1	Moderate, soft	6.00
7.5	Turbid, no nata formation	none	do	6.00
8.0	do	none	Turbid, no nata formation	none

* The yields of nata in this table and in all other tables were determined by water displacement in a 100-cc glass cylinder graduated to 1 cc.

concentrations which this organism can tolerate is so wide that it is not surprising at all to find it thrive in various rotting fruits of different degrees of sourness. No nata was formed at pH 3 or lower and at pH 8. Thicker nata formation was always observed in a nutrient medium with initial pH of 4.5 to 6. In coconut water medium, nata formation was observed to be optimum at pH 5 to 5.5. This was verified in many subsequent cultures in similar medium.

Influence of temperature on nata formation.—In this experiment the early indication of growth which was shown by the turbidity of solution was observed after 48 hours at temperatures of 20°, 25°, and 28° to 31°C (room temperature). No sign of growth was shown at 10°, 15°, 35°, and 40°C during the 48-hour period after inoculation.

The incipient formation of the white cartilaginous substance or nata was observed after 72 hours at room temperate (28-31°C) and after seven days at 20° and 25°C. At 35° and 40°C there was flocculent sedimentation as the culture aged but no nata formation was observed.

In Table 2 it is shown that nata was formed only at 20° and 25°C and at room temperature (28°–31°C). Room temperature is considered in this investigation as the optimum for nata formation since it gave consistently the highest average yield of the nata product. No nata formation was observed at 10°, 15°, 35°, and 40°C.

TABLE 2.—*Influence of temperature on nata formation in a synthetic medium of pH 5.*

Incubation temperature	Observation on nata formation	Average yield of nata after 15 days incubation
		Cm^2
10°C	No growth	0.00
15°C	do	0.00
20°C	Thin, soft	4.50
25°C	Moderate, firm	7.00
Room temperature (28 - 31°C)	Thick, firm	10.30
35°C	Turbid, no nata formation	0.00
40°C	do	0.00

Sugars suitable for nata formation.—In Table 3 it is shown that the nata organism is capable of subsisting on any of the sugars used in the experiment. However, the thickest formation of nata was induced by dextrose, followed closely by sucrose. The cultures containing galactose, lactose and maltose produced thin, soft nata. The concentration of dextrose and sucrose that produced the best formation of nata is 10 per cent. With galactose, lactose and maltose no substantial gain in thickness of nata formation is shown by increasing the concentration to 10 per cent. The tubes without sugar did not show any nata growth indicating that sugar is an indispensable nutrient for the growth of nata organism.

TABLE 3.—*Nata formation in different concentrations of some common sugars in yeast-extract-peptone-salt solution adjusted to pH 5.*

Sugar	Concentration	Observation on nata formation	Average yield of nata after 15 days incubation
	Per cent		Cm³
Control	0.0	No growth	0.00
Dextrose	2.0	Thick, firm	11.75
	6.0	do	17.00
	10.0	do	19.33
Galactose	2.0	Very thin, soft	1.35
	6.0	do	2.53
	10.0	do	3.33
Sucrose	2.0	Thick, firm	8.25
	6.0	do	14.75
	10.0	do	16.00
Lactose	2.0	Thin, soft	2.50
	6.0	do	2.50
	10.0	do	3.33
Maltose	2.0	Thin, soft	2.50
	6.0	do	3.13
	10.0	do	3.13

In the selection of sugar to be used as the source of carbon for nata organism, it may not be advantageous and profitable to use dextrose in commercial cultures although it can induce thick formation of nata because it is costly and is not available in large quantities in local markets. For large-scale culturing of nata organism, sucrose, a product of local sugar industry should be used because it is less costly and is obtainable here in any quantity at any time.

Nitrogenous substances favoring nata formation.—The results shown in Table 4 reveal that thick formation of nata is favored by the ammonium form of nitrogen. Dihydrogen ammonium phosphate gave the best result followed closely by ammonium sulfate. No decided advantage was gained by the addition of peptone. The nitrate form, whether it be sodium or potassium, gave no beneficial effect. In fact, both were toxic to the organism as neither induced nata formation when added to the synthetic basal medium. When either of these compounds was supplied to the coconut water medium, a retardation of the nata growth was shown as evidenced by lower yield obtained from cultures containing it than from the control.

In synthetic medium the batch that had no nitrogen showed a negligible growth, while in the coconut water medium, the cultures that contained no nitrogenous supplement showed a moderate formation of nata indicating the presence in the medium of an assimilable form of nitrogen which also induced nata formation. According to Miller(14) and Peters(17) the protein content of coconut water from the mature nut reaches to as much as 0.4 per cent and according to Palo and Lapuz(16) the suitableness of coconut water as medium for a great many microbes might be due partly to the presence in it of an assimilable form of protein. The accelerated growth of nata in coconut water medium could also be attributed to the growth-promoting factors which were reported to be present in it by Shantz and Steward (18, 19) and Miller, et al.(15)

In another experiment which was carried in sugared coconut skim milk medium purposely to determine further the stimulative influence of different concentrations of $NH_4H_2PO_4$ on nata formation, it was found that 0.5 per cent gave the highest yield of nata in both autoclaved and boiled series (Table 5). The incipient formation of nata was observed on the third day in batches containing 0.3, 0.5, 0.7, and 0.9 per cent and on the fourth day in those containing 0.1, 1.1, and 1.3 per cent and also in the controls (0 per cent). During the second week nata formation was faster in all those batches containing the introgenous salt than in those without it and resulted in the close similarity of thickness in all of them. After 15 days a very noticeable difference in the thickness of nata growth between the cultures containing $NH_4H_2PO_4$ and those without it (Plate 1, figs. 1 and 2) was observed.

TABLE 4.—*Influence of different forms of nitrogenous substances on nata yields.*

Basal medium	Nitrogen concentration*	Average yield of nata from cultures containing different nitrogenous supplements after 15 days incubation at room temperature (28—31°C)				
		Potassium nitrate (KNO_3)	Sodium nitrate ($NaNO_3$)	Ammonium sulfate $(NH_4)_2SO_4$	Ammonium phosphate ($NH_4H_2PO_4$)	Bactopeptone
	Per cent	*Cm*³	*Cm*³	*Cm*³	*Cm*³	*Cm*³
Synthetic: Potassium phosphate monobasic (KH_2PO_4) 0.4 gm Magnesium sulfate ($MgSO_4.7H_2O$) 0.4 gm Succinic acid (CH_3COOH) 1 gm Water to make 1,000 cc Sucrose ($C_{12}H_{22}O_{11}$) 100 gm pH 5	0.00	0.91	0.90	0.91	1.00	0.90
	0.10	0.00	0.00	1.50	3.00	0.70
	0.20	0.00	0.00	2.50	4.40	0.90
	0.30	0.00	0.00	3.66	6.00	1.25
	0.40	0.00	0.00	4.20	7.96	1.50
	0.50	0.00	0.00	7.50	8.30	2.00
	0.60	0.00	0.00	5.70	7.33	1.60
	0.70	0.00	0.00	4.50	6.16	1.61
	0.80	0.00	0.00	3.50	4.83	1.64
Coconut water: ** Sucrose ($C_{12}H_{22}O_{11}$) 100 gm Water to make 1,000 cc pH 5	0.00	4.00	4.30	4.20	4.16	4.33
	0.10	3.53	3.50	4.00	4.00	3.00
	0.20	3.16	3.26	4.20	4.50	3.50
	0.30	2.50	3.10	4.05	5.00	3.83
	0.40	2.00	2.50	6.50	6.60	4.33
	0.50	1.50	2.00	8.00	3.16	5.00
	0.60	1.20	1.50	6.60	7.00	4.50
	0.70	0.90	0.95	3.50	5.33	3.66
	0.80	0.50	0.70	4.16	4.50	3.50

* Computed on the basis of the nitrogen content of the nitrogenous substance.
** Protein content of coconut water, 0.1 per cent.

It is also shown in Table 5 that as the concentration of $NH_4H_2PO_4$ is increased from 0.1 to 0.5 per cent of the yield of nata in the two series mentioned above is correspondingly increased but on further increasing it up to 1.3 per cent the yield gradually decreased. Evidently, the optimum concentration is 0.5 per cent. The use, therefore, of $NH_4H_2PO_4$ as a nutrient ingredient of medium for nata organism is distinctly advantageous and very beneficial since it not only supplies the nitrogen needs of the nata organism but also stimulates thicker formation of the cartilaginous substance known as nata.

TABLE 5.—*Effect of varying the concentration of dihydrogen ammonium phosphate in sugared coconut skim milk*[a] *medium at pH 5 on nata formation.*

Concentration of ammonium phosphate ($NH_4H_2PO_4$)	Average yield of raw nata from 400-cc cultures in autoclave-sterilized coconut skim milk medium	Average yield of raw nata from 400-cc cultures in coconut skim milk medium subjected to boiling temperature for five minutes
Per cent	*gm*	*gm*
0.0	165.0	107.60
0.1	174.2	141.85
0.3	177.6	148.20
0.5	184.1	168.70
0.7	181.6	174.20
0.9	176.5	173.70
1.1	176.3	163.90
1.3	175.7	154.20

[a] Protein content of coconut skim milk, 1.80 per cent.

MORPHOLOGICAL AND CULTURAL FEATURES OF THE NATA ORGANISM

MORPHOLOGICAL CHARACTERISTICS

The nata organism is a rod-shaped nonmotile, Gram-negative bacterium which occurs singly and in chains. The individual cells which are enveloped by a gelatinous material measure 2 microns long and 0.6 micron wide. Cells from older cultures are longer, measuring usually from 4 to 9 or more microns. Fusion of the gelatinous envelopes form a continuous mass of cartilaginous tissue. A thin stained section of the membrane shows a jumbled pattern of bacterial cells and chains embedded within the cartilaginous tissue (Plate 2).

CULTURAL CHARACTERISTICS

Agar colonies.—On acidified tomato-peptone-yeast-sucrose agar where normal growth of the nata organism is always observed, the colonies become visible after 72 hours. Fully developed colonies are circular, opaque, raised, light brown, tough, and with smooth glistening surface and unbroken margin. They measure between 1 to 2 or more millimeters. Being tough, it is difficult to tear the colony apart. When the colony is touched with a glowing platinum needle or loop, it sticks to it and can be transferred whole into another tube of culture medium.

Agar slant.—On tomato-peptone-yeast-sucrose agar slant the growth along the line of inoculation is filiform and becomes tough with age.

Growth in liquid medium.—The early sign of growth is shown by turbidity of the saccharine medium after 24 hours. After 36 to 48 hours a translucent film forms on the surface. It gradually thickens into a cartilaginous tissue. When agitated, it sinks and another layer forms on the surface. Under favorable conditions the cartilaginous growth may thicken to 2 or more inches in one month.

PHYSIOLOGICAL CHARACTERISTICS[9]

Fermentation of common sugar.—Moderate growth of the selected nata organism was observed in all the inoculated tubes containing the basal solution[10] and the test sugars (arabinose, galactose, glucose, sucrose, and maltose) but acid formation was observed only in the tubes containing glucose as indicated by the change of color of the culture solution from purple to yellow.[11] There was no sign of growth of the nata organism in the control lots consisting of a set of inoculated tubes containing the basal solution without the test sugar and another set of uninoculated tubes containing both the basal solution and the test substance.

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[9] The procedure for all biochemical tests herein discussed are in accordance with those adopted by the Committee on Bacteriological Technique of the Society of American Bacteriologists.(6)

[10] Basal solution: $MgSO_4.7H_2O$, 0.4 gm; K_2HPO_4, 0.4 gm; $NH_4H_2PO_4$, 1 gm; succinic acid $(CH_2COOH)_2$, 1 gm; water, 1.000 cc.

[11] Brom cresol purple, the indicator used in this study was prepared by dissolving 50 mg of this substance in 100-cc alcohol. One cc of the alcoholic solution was added to 1,000 ml of the basal solution at pH 5.

Fermentation of common alcohols.—Moderate growth of the nata organism was observed in the inoculated tubes containing the synthetic basal medium shown in Table 4 and in those containing the same medium and 1-per cent ethyl, methyl, or propyl alcohol or glycol (ethylene alcohol). No growth of any microorganism was noted in the uninoculated control tubes and in the inoculated ones containing 3- and 5-per cent ethyl, methyl and propyl alcohols and glycol and in 1-, 3-, and 5-per cent butyl alcohol. Acid production as shown by a change of the color of the solution from purple to yellow was observed only in the tubes containing the 1-per cent ethyl and propyl alcohols ond glycol where the growth of nata organism was very evident.

Action on Bacto-litmus milk.—No curdling of litmus milk and no production of acid was observed even up to ten days.

Indol production test.—No indole was detected in 24-, 48-, and 72-hour-old cultures of the nata organism in tryptone broth. Its presence in the control tubes containing *Escherichia* coli (Migula) Castellani and Charmer, a well-known indole-producing microbe was markedly shown.

Nitrate reduction test.—No reduction of nitrate to nitrite was observed in cultures of nata organism in nitrate broth. Positive evidence of the presence of nitrite was shown in tubes inoculated with *E. coli.*

Production of catalase.—The nata organism was observed to be weakly catalase-positive. A pronounced production of catalase was shown by *E. coli* with which the nata organism was compared.

Oxidation of acetic acid.—Growth, which was characterized by a thin opaque film on the surface of the tomato-peptone-yeast-sucrose solution was observed in all the inoculated acetic and hydrochloric acid tubes after 24 hours of inoculation with nata organism. The film became thicker after 48 and 72 hours. The liquid beneath the film in each tube remained clear. No film was formed in all the uninoculated tubes. Upon the addition of calcium hydroxide in all tubes, turbidity was observed only in the inoculated acetic acid tubes indicating the presence of carbon dioxide after an incubation of 24, 48, and 72 hours. No turbidity was observed in the uninoculated tubes and in the inoculated hydrochloric acid tubes. These results showed that acetic acid was oxidized into CO_2 and water by the nata organism.

THE CELLULOSE REACTION OF NATA

In the preceding discussion it is revealed that nata is formed definitely by a bacillus and not by a dextran-forming coccus as reported by Adriano, et al.,(1) Africa,(2) and Alaban.(3) This being the case the cartilaginous substance known locally as nata might be composed mainly of a polysaccharide different from dextran. The positive cellulose reaction obtained in the test described in the preceding discussion showed that the nata organism is not a dextran-forming organism.

IDENTITY

Following the system of classification of the bacteria into families, tribes, and genera in Bergey's Manual of Determinative Bacteriology(6) the nata organism under study, being a heterotrophic, Gram-negative rod should belong to the family (Pseudomonadaceæ under which are recognized two tribes; namely, Pseudomonadeæ, the tribe comprising six genera, one of which is *Acetobacter,* the genus described to be nonmotile or motile by means of polar flagella and to possess the ability to oxidize alcohol to acetic acid. Since the nata organism is capable of producing acetic acid in a medium containing alcohol it should, therefore, be regarded as one belonging to this genus.

By running through the key for the identification of the species of *Acetobacter* it is noted that there are two groups, the first consisting of species capable of oxidizing acetic acid to carbon dioxide and water and the second comprising species that do not have this ability. As discussed ahead relevant to this subject the nata organism should belong to the first group under which five species; namely, *Acetobacter aceti, A. xylinum, A. rancens, A. pasteurianum,* and *A. kuetzingianum* have been recognized.

The ability of nata organism to utilize ammonium salts as a sole source of nitrogen places it under *A. aceti* according to Bergey's Manual of Determinative Bacteriology(6) but by virtue of the fact that it forms thick zoogleal membrane with cellulose reaction on the surface of suitable sugary solutions it can also be considered as close to *A. xylinum,* a "vinegar plant" which was first observed by Brown(7) to produce cellulose in a medium consisting of dextrose and yeast water.

In their studies of the nutrition of *Acetobacter* species Hall, et al.,(9) report *A. aceti, A. acetigenum, A. acetosum, A. kuetzingianum, A. mobile, A. orleanense, A. peroxydans, A. turbidans, A. xylinoides,* and *A. xylinum* to produce satisfactory growth in glucose-salts medium in which the sole source of nitrogen was diammonium hydrogen phosphate, 0.25 per cent (w/v) and the sugar was at concentration of 2.6 per cent (w/v). This shows that *A. aceti* does not have the exclusive ability to utilize ammonium salts as a sole source of nitrogen. Several other species including *A. xylinum* possess similar activity.

On the basis of morphological, physiological, cultural and other characteristics of nata organism as compared with those of other species belonging to the genus *Acetobacter* the authors believe that the organism in question lies very close to *Acetobacter xylinum* (Brown) Holland.

It is incomprehensible that the nata bacterium described above as identical with the cellulose-forming *Acetobacter xylinum* should be considered as similar to the dextran-forming *L. mesenteroides.* The agar colonies of the nata bacterium which Alaban(3) described as having a rubberlike consistency are far from being similar to the soft and slimy, viscous, or mucoid consistency of those of *L. mesenteroides.*

By comparing the nata organism with *L. mesenteroides* described by McCleskey, et al.,(12) it was noted that while they are alike in their ability to ferment arabinose, galactose, glucose, maltose, and sucrose they greatly differ in several other respects. These authors describe the shape of the four types of *L. mesenteroides* generally as oval or spherical and their size as 0.5 to 0.7 microns by 0.7 to 1.2 microns. The nata organism under study is distinctly rod-shaped measuring on the average 0.6 by 2 microns with tendency to lengthen to 4 to 9 or more microns in old cultures. In liquid saccharine media in which favorable growth occurs, the nata organism produces on the surface a translucent film which gradually develops into a thick cartilaginous substance of cellulose reaction while *L. mesenteroides* transforms similar medium into a slimy or mucoid mass consisting chiefly of dextran. This was our experience in connection with the local cabbage strain of *L. mesenteroides* studied by Arcega and Arguelles.(5)

Owing, therefore, to its close similarity to *Acetobacter xylinum* (Brown) Holland,[12] the nata organism under study will be referred to as such in this paper and in any subsequent study involving it.

SUMMARY

1. Nata, a cartilaginous substance formed by a species of acetic acid-forming group of bacteria in saccharine solutions, when produced in coconut water, coconut skim milk and fruit-juice media and processed into a desser, is now a popular Filipino delicacy. Its production from coconut water medium, in which many people have taken an interest, is gradually taking a role of importance in the coconut industry because coconut water, which goes to waste in large quantities in places where copra is made, can be utilized.

2. Cultural requirements for the formation of nata: nutrient medium, preferably one containing an ingredient with growth-promoting factors such as those present in coocnut water; temperature, about 28°C; pH 5 to 5.5; nitrogen source, ammonium salts preferably monobasic ammonium phosphate ($NH_4H_2PO_4$); carbon source, glucose and sucrose.

3. Nata is formed by an organism which according to its morphological, cultural and biochemical characteristics was found to be identical with *Acetobacter xylium* (Brown) Holland. This organism is rod-shaped, 2 microns long, usually occurring singly or in chains, nonmotile and Gram-negative. It forms acid from glucose, ethyl and propyl alcohols and glycol; weakly catalase-positive; litmus milk, not changed; indole not formed; nitrate not reduced; oxidizes acetic acid to carbon dioxide and water. Its distinctive character is its ability to form a cartiliginous membrane which holds the cells together and responds positively to a test for cellulose.

ACKNOWLEDGMENT

Sincere appreciation is expressed to Mrs. Luz Baens-Arcega, Director of the Biological Research Center, National Institute of

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[12] Identity of nata organism as *Acetobacter xylinum* was mentioned by the third author in the Proceedings of the PHILAAS Eleventh Annual Convention. Sci. Quart., Nos. 1-4 4 (1962) 15, 40.

Science and Technology, for her interest and helpful advice in the course of this project and to Mr. Martin S. Celino, Agricultural Research Consultant and Head, Agricultural Services Department, Philippine Coconut Administration, Diliman, Quezon City, for reviewing the manuscript.

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ILLUSTRATIONS

[The pictures in Plates 1 and 2 were taken by Dr. Clodualdo Angbengco, Dentist and Scientific Photographer, National Institute of Science and Technology.]

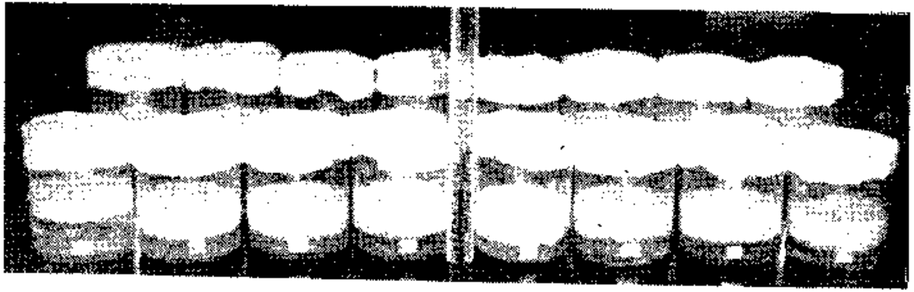
PLATE 1

FIG. 1. Fifteen-day-old cultures of nata organism showing the effect of different concentrations of $NH_4H_2PO_4$ on nata formation. Left to right 0.0 (control), 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, and 1.3 per cent.

2. A close-up of jars 0.0, 0.1, 0.5, and 0.7 per cent as shown in Fig. 1.

PLATE 2

Photomicrograph of a stained thin section of nata showing a confused mass of rod-shaped bacterial cells and crisscross chains of cells; about × 1800.



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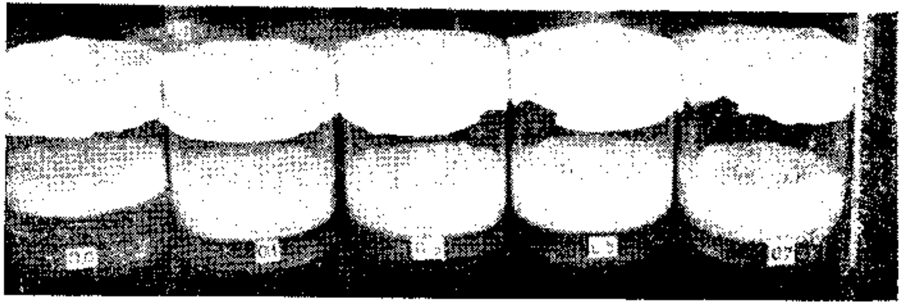

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PLATE 1.

PLATE 2.

A STUDY ON AN ACETIC ACID-FORMING BACTERIAL ISOLATE AND FACTORS INFLUENCING ITS GROWTH AND PRODUCTION OF ACETIC ACID OR VINEGAR FROM ALCOHOLIC MEDIUM

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THREE PLATES

Vinegar-making in the Philippines is, from the scientific viewpoint, an undeveloped industry as many manufacturers still resort to natural fermentation in the successive conversion of starchy or sugary raw materials first to wine and subsequently to vinegar. The wild yeasts and acetic acid bacteria which may be present in certain fruits may bring about spontaneous fermentation of the sugary juice but the manufacturer should not depend on chance alone and should use viable cultures of the selected pure strains of alcohol yeast and acetic acid bacteria in order to ensure production of vinegar with desirable flavor and aroma.

The Philippines, being a tropical country, abounds in sugary fruits which can be used as raw materials for vinegar manufacture. Surplus, blemished and overripe fruits classified as inferior, and therefore, cannot readily be disposed of in the market, can be utilized in making vinegar of good quality at nominal cost. Since practically any fruit juice or any agricultural byproduct and waste material containiing fermentable sugar can be utilized in vinegar-making, this investigation may be considered of special economic significance in regions where fruits and other sugary raw materials are plentiful as its success may stimulate the development in those areas of a profitable home industry. A year's supply of vinegar for use in the home can easily be made from surplus fruits. In copra-making regions the enormous amount of coconut water which goes to waste can be converted to vinegar of good quality.

There are no available statistics on annual production and consumption of vinegar in the Philippines. Of the several kinds of vinegar sold in the markets and stores and by street peddlers the three common ones are: (*a*) the so-called "sukang puti"

(Tagalog) or "Paombong" vinegar produced from sugary saps, known as "tuba" (Tagalog), of palms — nipa (*Nypha fruticans* Wurmb.), coconut (*Cocos nucifera* Linn.), "buri" (*Corypha elata* Roxb.), and "kaong" [*Arenga pinnata* (Wurmb.) Merr.]; (*b*) sugar vinegar or "sukang Iloco" (Tagalog) made from sugar cane juice, sweet molasses or sugar syrup; and (*c*) Del Monte vinegar manufactured from pineapple juice. A survey of vinegar consumption of 100 families in Manila and suburbs and in the rural areas of San Jose, Batangas Province and Imus, Cavite Province shows it to vary from 0.2 to 2 liters or an average of 0.63 liter per family per week or 32.76 liters per year. This excludes the consumption in restaurants, hotels and in food pickling plants. Based on these figures and considering that the Philippines is inhabited, as in 1965, by 5,548,027 families[1] the annual consumption of vinegar in this country should have reached a stupendous amount of 181,753,364 liters worth ₱27,263,004.60 if it were all "Paombong" vinegar, the commonest and cheapest kind of vinegar, with acetic acid content of about 2 to 2.91 per cent or an average of 2.5 per cent, which is peddled elsewhere in the Philippines at usually ₱0.15 a liter.

The preceding discourse shows various openings for commercial venture in vinegar production. It also offers opportunities for more and greater utilization of the sugary raw materials and saccharine by-products which abound in many districts in this country. The investigation discussed, hereunder, is designed primarily to acquire a superior strain of acetic acid bacterium adaptable for use in vinegar manufacture under existing local conditions.

MATERIALS AND METHODS

Isolations of acetic acid bacteria.—Ninety-five samples consisting of rotting ripe sugary fruits, fermenting palm sap and raw vinegar collected from Manila markets and several towns in nearby provinces were used as materials from which the vinegar bacteria were isolated. Isolation was made by following the pour-plate technique of Pelczar and Reid.(8) In the case of fruits, bits of the fleshy or pulpy part were first introduced into 400 ml paper-covered, wide-mouthed bottles containing sterile tomato-peptone-sucrose salts broth, a nutrient medium developed by Palo and Lapuz(7) for culturing *Streptococcus crystalloides* Palo and Lapuz and then after a week, platings from the pellicular growth were made on tomato-peptone-sucrose-salts agar. Vigorously growing colonies with light brown color and butyrous consistency which developed usually after an incubation period of four to seven days were marked and transfers were made from them to freshly-prepared tomato-peptone-sucrose-salts agar slants.

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[1] Supplied by the Statistical Division, National Economic Council of the Philippines, U.P. Alumni Building, Padre Faura, Manila.

Screening of isolates and selection of acetic acid-producing strain adaptable for home manufacture of vinegar.—The acetic acid-producing capacity of each of the 115 isolates retrieved from various sources mentioned above was tested in sterilized, paper-covered, wide-mouthed 400-ml bottles containing 6-per cent ethyl alcohol in Janke's medium [2] used by Coppin(5) for propagating vinegar bacteria. After determining the initial titrable acidity using phenolphthalein as the indicator the culture was incubated for 30 days at room temperature. Increase of acidity was determined by titrating the culture with N/10 sodium hydroxide solution. This procedure enabled the authors to determine and discard isolates which were incapable of producing significant increase in titrable acidity and to narrow the screening to only those which were able to produce appreciable amounts of acetic acid. The eight isolates which were capable of increasing the acidity to more than 3 per cent were selected and further tested in Junke's medium containing increasing concentrations (2, 4, 6, 8, and 10 per cent) of ethyl alcohol in Janke's medium and in yeast-fermented banana medium [3] with alcohol

[2] Formula of Janke's medium:

Monobasic potassium phosphate (KH_2PO_4)	0.4 gm
Dihydrogen ammonium phosphate ($NH_4H_2PO_4$)	1.0 gm
Magnesium sulfate ($MgSO_4.7H_2O$)	0.4 gm
Succinic acid ($HO_2CCH_2CH_2CO_2H$)	1.0 gm
Glycerine	5.0 gm
Distilled water	1,000.0 ml

[3] Banana medium was prepared by mixing 20 gm of mashed fleshy part of ripe "buñgulan" banana (*Musa sapientum* Linn. var. *suaveolens* Blanco) and 80 ml of 15-per cent solution of refined cane sugar in 400 ml wide-mouthed bottles. After pasteurizing the mixture for 30 minutes at 60° to 65°C it was inoculated with wine yeast (*Saccharomyces cerevisiæ* var. *ellipsoideus*) and allowed to ferment for five to seven days. The fermented mash was strained through cheese cloth. The filtrate was pasteurized at 60° to 65°C for 20 minutes to kill the yeast. After separating the yeast by centrifuging, the alcohol content of the fermented liquor was determined by means of a Lefco Ebulliometer.

content adjusted to 8 and 10 per cent. The isolate (No. 67-B) from a rotting mabolo (*Diospyros discolor* Willd.) fruit which gave the highest acetic acid-producing efficiency in 6 and 8 per cent ethyl alcohol in Janke's medium and in 8- and 10-per cent ethyl alcohol in fermented banana medium (Tables 1 and 2) was selected and used singly in all succeeding studies on its characterization and cultural requirements for the production of vinegar of high acidity.

Studies on cultural requirements for acetic acid production by the selected mabolo isolate.—All experiments described hereunder were carried in 400-ml wide-mouthed culture bottles each containing 100 ml of Janke's medium. Sterilization was done by autoclaving the culture bottles with the medium for 20 minutes at 15 pounds pressure per square inch. In experimental batches in which ethyl alcohol was necessary it was always added in concentration of 10 per cent into the sterilized medium which had been cooled down to room temperature. The incubation period was fixed at 30 days. The inoculant consisted of an actively-growing 48-hour-old culture of the mabolo bacterium in Janke's medium. The initial final titrable acidity of the culture was determined by titrating it with N/10 sodium hydroxide using phenolphthalein as indicator.

To determine the influence of pH on growth and acetic acid production the mabolo organism was grown in alcoholized Janke's medium adjusted with the aid of Beckman Zeromatic pH Meter to pH levels ranging from 2 to 10 at intervals of 0.5 (Table 3).

An experiment to determine the effect of temperature on its growth and capacity for acetic acid production was conducted by culturing the mabolo organism in alcoholized Janke's medium at pH 5 at controlled temperature of 10, 15, 20, 25, 35, and 40°C; at room temperatures (28°–32°C); and outdoor temperatures [diurnal (26.6°–40°C); nocturnal (23°–30°)].

To determine the suitable sources of nitrogen for growth and acetic acid production by the mabolo bacterium six nitrogenous substances; namely, yeast extract, bacto peptone, dihydrogen ammonium phosphate, ammonium sulfate, sodium nitrate and potassium nitrate were used. Each of these substances was added to the nitrogen-free Janke's medium in various amounts computed to contain nitrogen ranging, in concentration, from 0.01 to 0.08 per cent. After autoclaving the nutrient preparation and cooling it down to room temperature, it was alcoholized and adjusted to pH 5 and then inoculated with the mabolo strain of vinegar bacterium. An alcoholized nitrogen-free Janke's medium at pH 5 which was inoculated with the same organism was used as control.

Characterization and identification of the mabolo strain of vinegar bacterium.—The morphological, cultural and physiological properties of the selected mabolo bacterium were studied by following the procedures adopted by the Committee on Bacteriological Technic of the Society of American Bacteriologists(4) and the bacteriological methods of Clark and Halvorson(3) of the Department of Bacteriology, University of Illinois, Urbana, Illinois.

Owing to the absence of type and authenticated cultures of acetic acid bacteria in the Biological Research Center microbial culture collection with which the mabolo organism could be compared its identity would be difficult to determine.

To determine its specific identity, this organism was sent to Professor M. Aschner of Technion-Israel Institute of Technology, Haifa, Israel.

RESULTS AND DISCUSSION

The results of screening of 115 bacterial isolates for their acetic acid-producing capacity in 6-per cent ethyl alcohol in Janke's medium show that 60 of them were nonacid producers and 55 were acid producers. Of the acid producers 47 produced less than 2-per cent acetic acid and only eight were able to produce more than 3-per cent acetic acid. On further testing the last eight isolates in varying concentrations of ethyl alcohol in Janke's medium (Table 1) and in banana juice medium (Table 2) it was observed that as the concentration of the alcohol was increased from 2 to 10 per cent the yield of acetic acid was correspondingly increased except in three isolates (18, 86-A, and 86-B) in which case maximum yield was obtained at 8 per cent. Isolate 67-B from a rotting mabolo fruit which produced the highest yield of acetic acid in 10-per cent ethyl alcohol and proved to be the most efficient of all the isolates at 8-per cent ethyl alcohol was selected as the lone acetic acid-producing isolate to which all of the subsequent studies were centered.

Effect of initial pH on growth and acetic acid production by the selected strain of vinegar bacterium.—The results shown in Table 3 indicate that the mabolo bacterium can tolerate a wide range of initial pH. Early signs of pellicular growth occurred

TABLE 1.—*Acetic acid production by eight selected isolates in different concentrations of ethyl alcohol in Janke's medium with intial titrable acidity equivalent to* 0.0301 *N.*

Isolate No.	Alcohol concentration	Titrable acidity, normality, after 30 days incubation at room temperature (28°—32°C)	Increase in titrable acidity, normality	Increase of acidity as acetic acid	Efficiency*
				Per cent	*Per cent*
18	0.0	0.0301	0.0000	0.0000	00.00
	2.0	0.1168	0.0867	0.5202	20.00
	4.0	0.2905	0.2604	1.5624	30.04
	6.0	0.6241	0.5940	3.5640	43.69
	8.0	0.9591	0.9290	5.5740	53.59
	10.0	0.7810	0.7509	4.5054	34.65
19	0.0	0.0236	0.0000	0.0000	00.00
	2.0	0.0448	0.0147	0.0882	3.39
	4.0	0.3333	0.3032	1.8192	34.98
	6.0	0.5540	0.5240	3.1440	40.30
	8.0	0.7356	0.7055	4.2330	40.70
	10.0	0.9138	0.8837	5.3022	40.78
22	0.0	0.0301	0.0000	0.0000	00.00
	2.0	0.0891	0.0590	0.3541	13.61
	4.0	0.3568	0.3267	1.9602	37.69
	6.0	0.5621	0.5320	3.1920	40.92
	8.0	0.7595	0.7294	4.3764	42.08
	10.0	0.9826	0.9225	5.7150	43.96
63	0.0	0.0323	0.0022	0.0132	00.00
	2.0	0.0391	0.0090	0.0540	2.07
	4.0	0.0460	0.0159	0.0954	1.83
	6.0	0.5321	0.5020	3.0120	33.61
	8.0	0.8385	0.8084	4.8504	46.63
	10.0	0.8386	0.8085	4.8510	37.31
67-B	0.0	0.0286	0.0000	0.0000	00.00
	2.0	0.1463	0.1162	0.6972	26.81
	4.0	0.4263	0.3935	2.3610	45.40
	6.0	0.7041	0.6740	4.0440	50.55
	8.0	0.9720	0.9419	5.6514	54.34
	10.0	1.0293	0.9992	5.9952	46.11
77	0.0	0.0301	0.0000	0.0000	00.00
	2.0	0.0395	0.0094	0.0564	2.16
	4.0	0.2136	0.1835	1.1010	21.17
	6.0	0.5700	0.5399	3.2394	41.53
	8.0	0.8868	0.8567	5.1402	49.42
	10.0	0.9081	0.8780	5.2680	40.52
86-A	0.0	0.0301	0.0000	0.0000	00.00
	2.0	0.0433	0.0132	0.0792	3.04
	4.0	0.1621	0.1320	0.7920	13.30
	6.0	0.6095	0.5794	3.4764	44.55
	8.0	0.9710	0.9409	5.6454	54.28
	10.0	0.7458	0.7157	4.2942	33.26
86-B	0.0	0.0301	0.0000	0.0000	00.00
	2.0	0.0433	0.0132	0.0792	3.04
	4.0	0.1666	0.1365	0.8190	15.73
	6.0	0.5625	0.5324	3.1944	40.95
	8.0	0.9671	0.8370	5.0220	48.28
	10.0	0.7458	0.7157	4.2942	33.03

* The acetic acid-producing efficiency of a bacterial isolate $= \dfrac{\text{Actual yield of acetic acid}}{\text{Theoretical yield of acetic acid}} \times$ 100 per cent

TABLE 2.—*Vinegar production by eight selected isolates of acetic acid bacteria in 8- and 10-per cent ethyl alcohol* in banana medium with initial titrable acidity equivalent to* 0.0421 *N.*

Isolate No.	Alcohol concentration	Titrable acidity, normality, after 30 days incubation at room temperature (28°—32°C)	Increase in titrable acidity, normality	Increase of acidity as acetic acid	Efficiency**
				Per cent	*Per cent*
18	8.0	0.5701	0.5280	3.1680	30.46
	10.0	0.8278	0.7857	4.7142	36.26
19	8.0	0.8176	0.7755	4.6530	44.74
	10.0	0.9221	0.8800	5.2800	40.61
22	8.0	0.6710	0.6289	3.7734	36.28
	10.0	0.9691	0.9270	5.5620	42.77
63	8.0	0.8443	0.8012	4.8072	46.22
	10.0	0.8803	0.8382	5.0292	36.68
67-B	8.0	0.9988	0.9567	5.7402	55.19
	10.0	1.0436	1.0015	6.0090	46.22
77	8.0	0.7710	0.7289	4.3738	42.05
	10.0	0.9003	0.8582	5.1492	39.60
86-A	8.0	0.6586	0.6165	3.6990	35.55
	10.0	0.8908	0.8487	5.0922	39.17
86-B	8.0	0.6751	0.6330	3.7980	36.51
	10.0	0.7650	0.7229	4.3374	33.36

* Original alcohol content of fermented banana medium, 9.7276 per cent by volume.
** Efficiency (As in footnote of Table 1).

at pH 4 to 5.5 after a week's incubation at room temperature. Neither visible growth nor increase of acidity was observed at pH levels below 3 and above 8. Optimum acetic acid production was observed in the medium with an initial pH of 5. With its ability to thrive in a wide range of pH it may not be surprising to find growth of the mabolo bacterium in several kinds of fruits of different degrees of sourness or various stages of microbial decomposition.

Relation of temperature to growth and acetic acid production by the mabolo bacterium.—Early signs of growth indicated by the formation of a thin friable pellicle was observed on the second week at temperatures of 20°, 25°, 28°–32° (room temperature), 35°C and outdoor temperatures (23°–40°C). Scanty growth was observed at 10° and 40°C. Slight growth was shown at 15°C. In Table 4, it is shown that growth and acetic acid production by the mabolo bacterium occurred in temperatures ranging from 15° to 35°C with optimum occurring within limits of 28° to 32°C

TABLE 3.—*Relation of initial pH to growth and acetic acid production by the selected mabolo bacterium in 10-per cent ethyl alcohol in Janke's medium.*

Initial pH of medium	Initial titrable acidity, normality	Titrable acidity, normality, after 30 days incubation at room temperature (28°—32°C)	Increase in titrable acidity, normality	Increase of acidity as acetic acid	Appearance of growth
				Per cent	
2.0	0.0763	0.0763	0.0000	0.0000	No growth
2.5	0.0458	0.0458	0.0000	0.0000	do
3.0	0.0356	0.4516	0.4160	2.4960	Pellicle, slimy easily sinks if disturbed.
3.5	0.0310	0.7778	0.7468	4.4808	Pellicle, slimy, easily sinks if disturbed, ascending on wall of glass container; medium turbid
4.0	0.0255	0.9051	0.8796	5.2776	do
4.5	0.0230	0.9520	0.9290	5.5818	do
5.0	0.0201	0.9763	0.9562	5.7372	do
5.5	0.0168	0.9471	0.9303	5.5740	do
6.0	0.0146	0.8701	0.8555	5.1330	do
6.5	0.0123	0.7913	0.7790	4.6740	do
7.0	0.0050	0.6458	0.6408	3.8448	do
7.5	0.0026	0.4851	0.4825	2.8950	do
8.0	0.0000	0.2983	0.2983	1.7898	Thin, hardly visible film
8.5	0.0000	0.0100	0.0100	0.0700	do
9.0	0.0000	0.0000	0.0000	0.0000	Ring formation, clear medium
9.5	0.0000	0.0000	0.0000	0.0000	No growth
10.0	0.0000	0.0000	0.0000	0.0000	do

Table 4.—*Relation of temperature to growth and acetic acid production by the mabolo bacterium in 10-per cent ethyl alcohol in Janke's medium with an initial titrable acidity equivalent to 0.0200 N.*

Incubation temperature	Titrable acidity, normality, after 30 days incubation	Increase in titrable acidity, normality	Increase of acidity as acetic acid	Appearance of growth
°C			*Per cent*	
10	0.0913	0.0713	0.4278	Slight turbidity of medium
15	0.1956	0.1756	1.0536	Thin film, hardly visible
20	0.5923	0.5723	3.4338	Pellicle, heavy, slimy, ascending on wall of glass container, easily sinks if disturbed; medium slightly turbid.
25	0.9486	0.9286	5.5716	do
Room tempe- (28°—32°C	0.9696	0.9496	5.6976	do
35	0.5621	0.5421	3.2526	do
Outdoor temperatures (Nocturnal 23°—30.2°C; Diurnal 26.6°—40°C)	0.0913	0.0713	0.4278	Slight turbidity of medium
40	0.0909	0.0709	0.4254	do

(room temperature). Outdoor cultures at temperatures varying from 26.6° to 40°C (diurnal) and 23° to 30.2°C (nocturnal) showed only slight turbidity of the medium showing that growth and development of the mabolo bacterium was inhibited by exposure to direct rays of the sun or even to diffused sunlight. This result seems to substantiate an observation of Mitchell(6) who in connection with the inhibiting effect of sunlight on the organism states that "the vitality of the acetic acid bacteria is reduced when the organism is exposed to sunlight."

Growth and acetic acid production by the mabolo bacterium seems to be still close to normal at 25°C. As incubation temperatures were lowered from 20°C down to 10°C, growth and acetic acid production gradually declined until at 10°C they were almost nil. At 35°C the organism under study showed a marked decrease in acetic acid production and at 40°C its growth was so repressed that only a little amount of acid was formed.

Since the optimum temperatures for its growth and acetic acid production lies within limits of the uncontrolled room temperatures (28°–32°C) the mabolo organism divulged a characteristic feature which is very much desired in an organism to be used in large scale vinegar manufacture because its possession of such a property will not require equipment for the adjustment of temperature which might entail additional expense.

Nitrogenous substances influencing acetic acid production by the mabolo bacterium.—Good growth of the mabolo bacterium shown by copious pellicular formation was produced in all batches containing different nitrogenous substances whether they were organic, ammonium or nitrate forms. The nitrogen-free cultures did not produce any visible sign of growth.

Higher production of acetic acid, appeared to have been favored by the organic forms (yeast extract and bacto-peptone) and by the phosphatic form of ammonium compound (Table 5). According to Rao and Stokes(10) nitrogenous materials of biological origin like yeast extract and peptone are necessary for the growth of many species of acetic acid bacteria of the genus *Acetobacter*. The same authors(11) observed also that this group of bacteria could not grow well in "chemically defined media with ethanol as the sole source of carbon and energy" unless small amounts of yeast autolysate, peptone or other complex organic substances are added to the medium.

Going back to Table 5 it will be seen that the nitrogen concentration most favorable for acetic acid production by the mabolo bacterium is 0.02 per cent equivalent to 0.2 gram of available nitrogen present in the nitrogenous material per liter of the medium.

It is interesting to note that the only inorganic form of nitrogen which closely approximates yeast extract or bacto-peptone in stimulating acetic acid production by the mabolo bacterium is dihydrogen ammonium phosphate. Since this nitrogenous compound is relatively much cheaper than either of the above nitrogenous substances of biological origin, its use as a source of nitrogen for the mabolo bacterium to be used in vinegar production is believed to be more advantageous because it wll certainly lower the cost of production which may make possible the realization of substantial returns.

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TABLE 5.—*Effect of different nitrogenous sources on growth and acetic acid production by the mabolo strain of vinegar bacterium in 10-per cent ethyl alcohol in nitrogen-free Janke's medium*[1] *with an initial titrable acidity equivalent to* 0.0250 *N.*

Nitrogenous substance	Concentration of available nitrogen	Weight of nitrogenous substance per liter of nitrogen-free Janke's medium	Titrable acidity, normality, after 30 days incubation at room temperature (28—32°C)	Increase in titrable acidity, normality	Increase of acidity as acetic acid	Appearance of growth
	Per cent	*gm*			*Per cent*	
Yeast extract[2] (Baltimore Biological Lab.)	0.01	1.030	0.9765	0.9515	5.7090	Pellicle, slimy, cream-colored, easily sinks if disturbed, ascending on wall of glass container; medium turbid.
	0.02	2.060	1.0290	1.0040	6.0240	
	0.03	3.090	1.0031	0.9781	5.8686	
	0.04	4.120	0.8910	0.8660	5.1960	
	0.05	5.150	0.7858	0.7608	4.5648	
	0.06	6.180	0.6828	0.6578	3.9468	
Bacto-peptone[2] (Difco Lab.)	0.01	0.625	0.9888	0.9638	5.7828	do
	0.02	1.250	0.9988	0.9738	5.8428	
	0.03	1.875	0.9938	0.9688	5.8128	
	0.04	2.500	0.9536	0.9286	5.5716	
	0.05	3.125	0.9135	0.8885	5.3310	
	0.06	3.750	0.8833	0.8583	5.1498	
Dihydrogen ammonium phosphate, A. R. ($NH_4H_2PO_4$)	0.01	0.821	0.8615	0.8360	5.0160	Pellicle, slimy, white, easily sinks if disturbed, ascending on wall of glass container; medium turbid.
	0.02	1.642	0.9883	0.9633	5.7798	
	0.03	2.463	0.9763	0.9513	5.7078	
	0.04	3.284	0.9581	0.9331	5.5986	
	0.05	4.105	0.8555	0.8305	4.9830	
	0.06	4.926	0.7346	0.7096	4.2576	
Ammonium sulfate, A. R. $(NH_4)_2SO_4$	0.01	0.471	0.6683	0.6433	3.8598	do
	0.02	0.942	0.6925	0.6675	4.0050	
	0.03	1.413	0.7588	0.7338	4.4028	
	0.04	1.884	0.7588	0.7338	4.4028	
	0.05	2.355	0.7045	0.6795	4.0770	
	0.06	2.825	0.6561	0.6311	3.7866	
Sodium nitrate, Merck's Reagent ($NaNO_3$)	0.01	0.607	0.7255	0.7005	4.2030	do
	0.02	1.214	0.7285	0.7035	4.2210	
	0.03	1.821	0.7285	0.7035	4.2210	
	0.04	2.428	0.7941	0.7691	4.6146	
	0.05	3.035	0.8342	0.8092	4.8552	
	0.06	3.642	0.8458	0.8208	4.9348	
Potassium nitrate, 'Baker analyzed' reagent (KNO_3)	0.01	0.720	0.7371	0.7121	4.2726	do
	0.02	1.440	0.8695	0.8445	5.0670	
	0.03	2.161	0.7426	0.7176	4.3056	
	0.04	2.881	0.7206	0.6956	4.1736	
	0.05	3.602	0.7041	0.7691	4.0746	
	0.06	4.322	0.5641	0.5391	3.2346	
Control (Alcoholized nitrogen-free Janke's medium	0.00	0.000	0.0875	0.0625	0.3750	No cloudiness and sign of pellicular formation

[1] Nitrogen-free Janke's medium was prepared by omitting $NH_4H_2PO_4$ from Janke's medium whose composition is shown as footnote 2.

[2] Nitrogen content of yeast extract, 9.688 per cent; peptone, 16.22 per cent; as found by Improved Kjeldahl method described in Official Methods of Analysis — Association of Agricultural Chemists. (1)

THE MABOLO VINEGAR BACTERIUM

IDENTITY

In a letter, dated November 16, 1964, Professor M. Aschner of the Department of Food and Biotechnology, Technion-Israel Institute of Technology, Haifa, Israel, named the mabolo bacterium *Acetobacter rancens* Beijerinck var. *turbidans* Frateur. He described this bacterium briefly as follows: "The organism oxidizes alcohol to acetic acid with weak suboxidation of the acid formed. On 10-per cent glucose yeast extract agar it forms gluconic acid but not 5 keto-gluconate crystals. It oxidizes calcium lactate, but does not form mannitol, it produces turbidity in fluid media."

Other characteristic features of this organism which were studied in detail in the Biological Research Center laboratory are described hereunder.

MORPHOLOGICAL CHARACTERISTICS

The cells appear as non-motile short rods with rounded ends, occurring either singly, in pairs or in short chains (Plate 1, fig. 1). Cells from young cultures are Gram-negative; those from old ones are Gram-variable. Individual cells measure generally from 1 to 2 microns long and from 0.5 to 0.8 micron wide. A few cells which appear longer, measure from 3 to 6 or more microns.

Involution forms (Plate 1, fig. 2) have been frequently observed in old, highly acidic cultures. They are filamentous, swollen and sometimes distorted in shape. These abnormal cells seem to be the same as the dead or dying cells described by Stanier, et al.(12) On the other hand, Mitchell(6) states that abnormality of shape of bacterial cells is influenced by temperature and composition of the medium.

CULTURAL CHARACTERISTICS

Agar colonies.—On tomato-peptone-sucrose-salts agar, with 4-per cent ethyl alcohol, growth becomes visible after 24 to 48 hours appearing as tiny colonies with circular and glistening surface (Plate 2, fig. 1). Fully developed colonies are light brown, circular, entire, opaque, and convex. They measure from 1 to

3 or more mm. In plates of ethanol-calcium carbonate agar [4] the mabolo bacterium produces clear areas around the colonies (Plate 2, fig. 2) indicating formation of acetic acid which has caused dissolution of the fine, white calcium carbonate powder.

Agar slant.—On tomato-peptone-sucrose-salts agar slant the growth is filiform (Plate 3, fig. 1), light brown becoming darker with age.

Growth in liquid medium.—Growth becomes visible after 24 to 48 hours in liquid medium containing 4-per cent ethyl alcohol. The pellicle covers the entire surface of the liquid (Plate 3, fig. 2) usually after four days to a week. Slight agitation of the culture breaks the pellicular growth and causes it to sink to the bottom of the container. When the culture remains undisturbed for several days the pellicle thickens into a layer of growth 2- to 3-mm thick. The growth along the edge of the pellicle has a tendency, while still young, to creep up the moist wall of the container, a characteristic exhibited by several species of acetic acid bacteria. As the culture ages, the bacterial cells gradually settle to the bottom of the container.

PHYSIOLOGICAL CHARACTERISTICS

Action on carbohydrates and alcohols.—From the results shown in Table 6 it appears that the mabolo bacterium is incapable of fermenting a large variety of carbohydrates and alcohols. Of the ten different carbohydrates tested only glucose was fermented and of the 11 alcohols tried only ethyl, n-propyl, n-butyl and isobutyl alcohols and ethylene glycol underwent dissimilation. In all of these cases of fermentation, acid was formed as shown by a change of color of the indicator, brom-cresol purple, from purple to yellow but in no case was gas produced.

Action on milk.—No growth occurred and no acid was produced in litmus milk and plain skimmed milk inoculated with

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[4] Formula of ethanol-calcium carbonate agar developed by Prof. R. Wolfe of the Department of Microbiology, University of Illinois as shown in a Laboratory Guide prepared by him for Microbiology 309 class:

Yeast extract	10.0 gm
Calcium carbonate ($CaCO_3$)	10.0 gm
Tryptone	2.0 gm
Agar	20.0 gm
Distilled Water	1,000.0 ml

Cool sterilized medium down to 45°, add 7 ml of filter-sterilized 95-per cent ethanol per 100 ml and mix well.

TABLE 6.—*Fermentation of carbohydrates and related compounds.*

Fermentable substance	Reaction after 10 days of incubation at room temperature in a basal broth[1] at pH 7.	Gas production
	pH	
Monosaccharides:		
Glucose ($C_6H_{12}O_6$)	6.15	None
Arabinose [$CH_2OH(CHOH)_3CHO$]	7.00	do
Sorbose [$CH_2OH(CHOH)_3COCH_2OH$]	7.00	do
Disaccharides:		
Sucrose ($C_{12}H_{22}O_{11}$)	7.00	do
Lactose ($C_{12}H_{22}O_{11}.H_2O$)	7.00	do
Maltose ($C_{12}H_{22}O_{11}.H_2O$)	7.00	do
Polysaccharides:		
Raffinose ($C_{18}H_{32}O_{16}$)	7.00	do
Starch ($C_6H_{10}O_5$)x	7.00	do
Inulin ($C_6H_{10}O_5$)x	7.00	do
Glycogen ($C_6H_{10}O_5$)x	7.00	do
Alcohols:		
Methyl alcohol (CH_3OH)	7.00	do
Ethyl alcohol (C_2H_5OH)	4.70	do
n-Propyl alcohol ($CH_3CH_2CH_2OH$)	5.75	do
Isopropyl alcohol ($CH_3CHOHCH_3$)	7.00	do
n-Butyl alcohol ($CH_3CH_2CH_2CH_2OH$)	5.60	do
Isobutyl alcohol [$(CH_3)_2CHCH_2OH$]	5.55	do
Amyl alcohol ($CH_3CH_2CH_2CH_2CH_2OH$)	7.00	do
Ethylene glycol [$C_2H_4(OH)_2$]	5.15	do
Glycerol ($CH_2OHCHOHCH_2OH$)	7.00	do
Mannitol [$CH_2OH(CHOH)_4CH_2OH$]	7.00	do
Dulcitol [$CH_2OH(CHOH)_4CH_2OH$]	7.00	do
Controls:		
Inoculated medium with no fermentable substance	7.00	do
Uninoculated medium containing each of the fermentable substance	7.00	do

[1] Basal broth was prepared by omitting glycerine from Janke's medium and adding bromcresol purple as indicator and whose composition is shown as footnote 2.

the mabolo bacterium. The check organism, *Escherichia coli* (Migula) Castellani and Chalmers produced acid, curd and whey in the medium.

Liquefaction of gelatin.—All inoculated tubes of gelatin medium produced growth but when immersed in cold water after two, four, and seven days incubation at room temperature resolidified indicating that the organism was incapable of hydrolyzing proteins in gelatin to polypeptides and amino acids.

Hydrolysis of starch.—Blue color was shown after flooding the surface of the inoculated starch agar plates with Lugol's iodine solution indicating that the mabolo organism was not able to hydrolyze starch

Production of hydrogen sulfide.—No hydrogen sulfide was produced by the mabolo bacterium on iron-citrate agar. The well-known sulfide-forming, *Salmonella typhosa* (Zopf) White, which was simultaneously cultured on the same agar produced blackening of the slant.

Indole production.—Indole was not detected from cultures in tryptone broth but it was formed in check tubes containing *E. coli.*

Action on nitrates.—Nitrate was not reduced to nitrite by the mabolo bacterium. The same test produced positive result with *E. coli,* a nitrate-reducing organism.

Catalase production.—The mabolo bacterium was observed to be strongly catalase positive. The same result was obtained with *E. coli,* a strongly catalase-positive organism.

Oxygen requirements.—The organism was observed to grow only on the upper 3-mm column of an agar shake culture showing that the mabolo bacterium is an obligate ærobe.

Oxidation of acetic acid to carbon dioxide and water.—The mabolo organism is capable of weakly oxidizing acetic acid to carbon dioxide and water.

SUMMARY

1. The 115 isolates derived from rotting ripe sugary fruits, palm saps and raw vinegar which were screened for acetic acid production yielded eight strains capable of producing more than 3-per cent acetic acid from 6-per cent ethyl alcohol in Janke's medium. On further testing these eight isolates in varying alcohol concentrations in Janke's medium and in yeast-fermented banana medium, Isolate 67-B from a rotting mabolo (*Diospyros discolor* Willd.) fruit proved to be the best. Its acetic acid-producing efficiency of 54.34 and 55.19 per cent in 8-per cent ethyl alcohol obtained respectively in Janke's medium and in yeast-fermented banana medium was the highest. Highest acetic production was, however. obtained in 10-per cent ethyl alcohol in both media.

2. The mabolo batcerium was identified by Prof. A. Aschner of the Department of Food and Biotechnology, Technion-Israel Institute of Technology, Haifa, Israel as *Acetobacter rancens* (Beijerinck) var. *turbidans* Frateur.

3. Salient features of the mabolo bacterium are described as follows: Cells rod-shaped 1 to 2 microns long and 0.5 to 0.8 mi-

cron wide; occur singly or in short chains; non-motile; Gram-negative when young and Gram variable in older cultures; gelatin stab, no liquefaction; litmus milk, no growth; nitrate, not reduced; indole, not formed; starch, not hydrolyzed; hydrogen sulfide, not produced; obligately ærobic; strongly catalase-positive; acetic acid, weakly oxidized to carbon dioxide and water; forms acid but not gas from glucose, ethylene glycol and ethyl, n-propyl, n-butyl and iso-butyl alcohols.

4. Optimal conditions for growth and production of acetic acid: pH 5; temperature, 28° to 32°C (room temperature); nitrogen requirements preferably organic in nature and phosphatic form of ammonium salt; cool temperatures and sunlight, inhibitory to growth.

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ILLUSTRATIONS

[The photomicrographs in Plate 1 and the photographs in Plates 2 and 3 were taken by Mr. Nemesio Bercasio, Technical Photographer, Division of Documentation, NIST.]

PLATE 1

FIG. 1. The mabolo bacterium [*Acetobacter rancens* (Beijerinck) var. *turbidans* [(Frateur)] from a 48-hour-old culture on tomato-peptone-sucrose-salts agar, stained with crystal violet to show the short rods with rounded ends occurring in two's and in short chain; approximately, × 2,000.

2. Cells from four-week-old culture in yeast-fermented banana medium stained with crystal violet to show the involution forms; appropimately, × 2,500.

PLATE 2

FIG. 1. Showing fully-developed colonies of the mabolo bacterium from a week-old culture on tomato-peptone-sucrose-salts agar; approximately, × 7/10.

2. Showing colonies of the mabolo bacterium on ethanol-calcium carbonate agar surrounded with clear areas caused by the action of acetic acid produced by the mabolo organism on calcium carbonate; approximately, × 7/10.

PLATE 3

FIG. 1. On tomato-peptone-sucrose-salts agar slant showing the filiform type of growth; about, × ⅓.

2. Showing the copious pellicular growth in alcoholized tomato-peptone-sucrose-salts (*a*) and thinner formation of the surface growth in similar medium with alcohol (*b*); about, × 5/16.

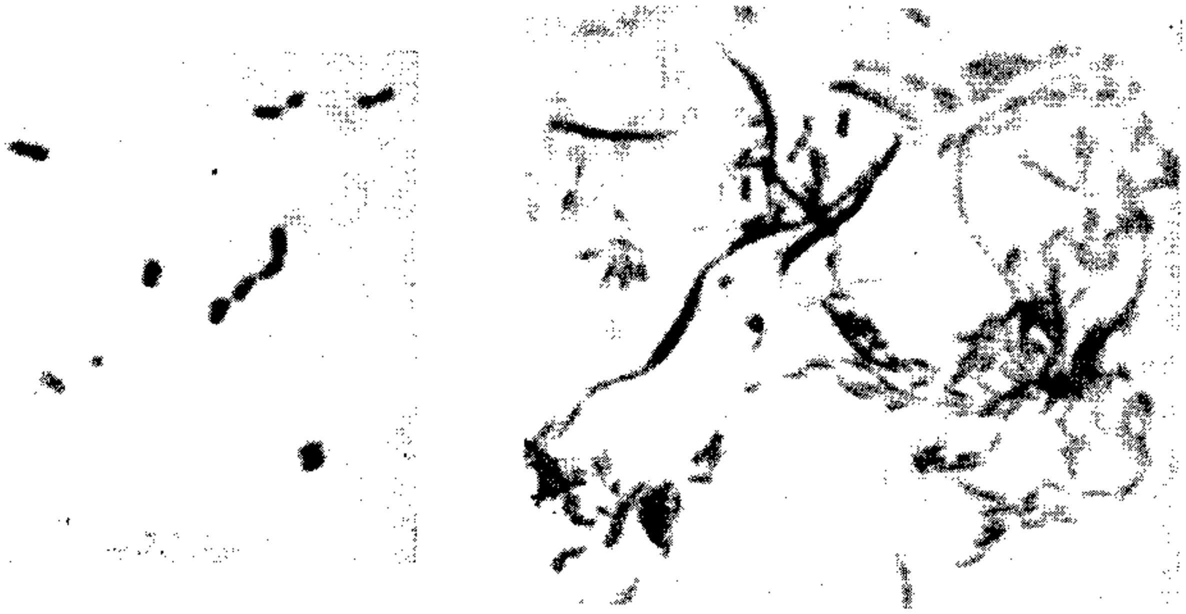

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PLATE 1.

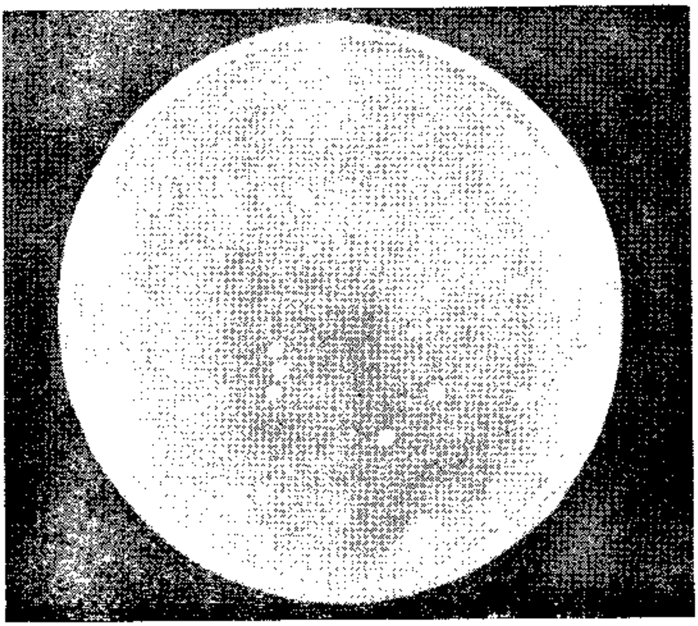

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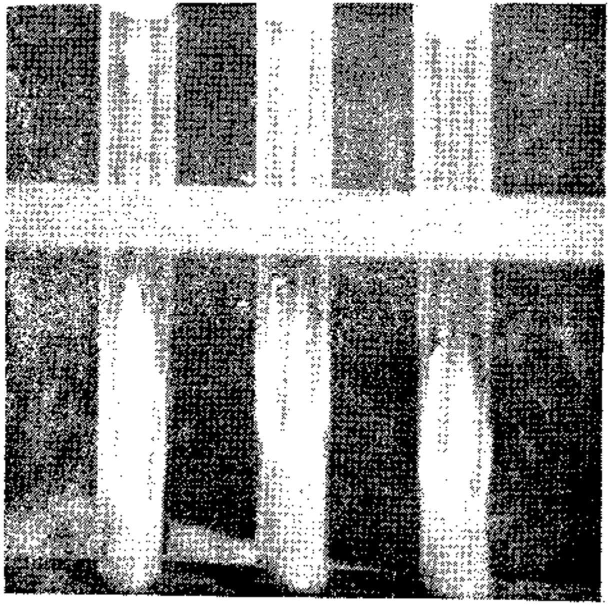
PLATE 2.



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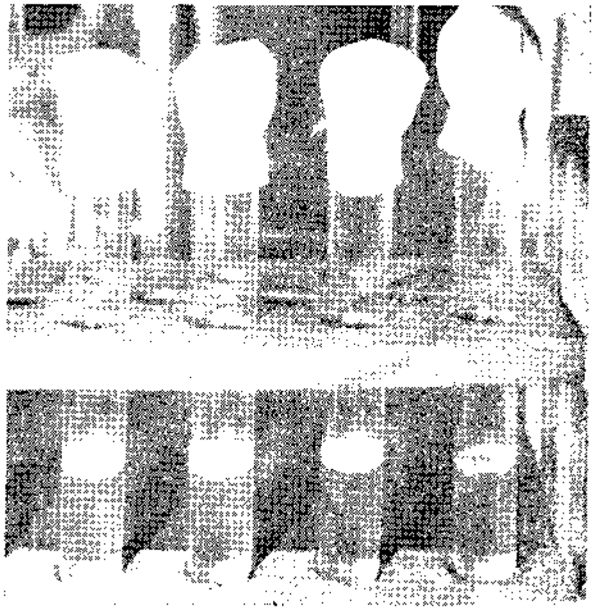

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a *b*

PLATE 3.

STUDIES ON THE PREPARATION OF "SOY" SAUCE FROM COCONUT PARING MEAL

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INTRODUCTION

Soy sauce, locally known as *toyo*, is a protein hydrolysate condiment which is a popular item in the Filipino diet because of its flavor-improving characteristic. The manufacture of this sauce dates back to ancient times and according to historians, the Chinese were the first to produce and use this food flavoring condiment.(4)

Soybean is the primary raw material in the commercial production of *toyo*. Since the Philippines is not a producer of soybean and because of its high market price, this study was conducted to determine the feasibility of substituting coconut paring meal in whole or in part in the manufacture of *toyo*.

Paring meal is a by-product of the desiccated coconut industry and is readily available in big amounts in desiccated coconut factories. Paring is the brownish coating of the coconut meat and is directly adjacent to the shell. At present, it is used mostly as animal feed. Its industrial possibilities, particularly, its utilization as food for human consumption has not been fully explored.

The proximate chemical composition of paring meal compares favorably with those of soybean and copra meal, except that the former has more protein. Since a condiment is not actually intended to be a protein source this deficiency should not be the basis for rejecting this cheaper raw material for sauce manufacture.

Voluminous literature on the manufacture of sauce from soy beans by fermentation mostly in oriental languages are available. There are also a number of publications on similar protein condiments prepared by direct acid hydrolysis. A procedure thought to have some advantage was reported by Lockwood and Smith.(6)

It is a combination of the acid hydrolysis and enzyme digestion method.

Numerous publications on soy sauce have been reported by Japanese workers. Studies on aroma and flavor of Japanese soy sauce made by Yokutsuka(12) indicated that the four major characteristics of Japanese soy sauce, which makes it different from other types produced in the Orient are: aroma, flavor, color, and stability.

In the Philippines some studies on the manufacture and composition of soy sauce and similar products have been reported. De la Cruz(3) made a study on the protein content of soy sauce in different mixtures prepared by mold-enzyme digestion.

Yenko and Arcega(11) reported on the utilization of rice flour as a substitute for wheat flour in the preparation of soy sauce by the enzyme hydrolysis method. Mendoza(7) worked on the utilization of copra meal in the preparation of coco "soy" sauce following the process of fermentation and harvesting used conventionally in the manufacture of Chinese soy sauce. The product was reported to be comparable to both the Chinese and Japanese soy sauces.

There are no reports of previous investigations on the utilization of paring meal in sauce manufacture. This industry-oriented research project was therefore undertaken to exploit this by-product in line with the NIST by-product industrialization of various food products from coconut. The quality of sauces prepared entirely or partly from soybeans or coconut paring meal using the acid hydrolysis and enzyme digestion methods, was determined.

MATERIALS AND METHODS

Paring meal, soybean and wheat flour were the basic raw materials used in the preparation of the experimental hydrolysate condiments by the acid hydrolysis and enzyme digestion methods. Paring meal was supplied by a desiccated coconut factory, while soybeans and wheat flour were purchased from the local market. Paring meal and soybean were analyzed for protein and moisture contents.

Acid-hydrolysis method.—Five trials were conducted, using the following proportion of materials: Trial 1, paring meal alone; Trial 2, paring meal and soybean in the proportion of 2:1; Trial

3, paring meal and soybean at 1:1 ratio; Trial 4, paring meal and soybean at 1:2 ratio; and Trial 5, soybean alone.

The following general process was followed in the preparation of the experimental acid hydrolysate condiments:

Fifty grams each of the above prepared materials were mixed thoroughly with an equal weight of toasted wheat flour and transferred into a 1,000-ml round bottom flask. One hundred ml of water was added to the mixtures to produce a pasty mass. A 400-ml volume of 20-per cent hydrochloric acid was poured into the mixtures. To the round bottom flasks, air condensers of about 30 inches long were connected and the mixtures allowed to boil under controlled temperature for 16 hours. Minor (8) reported that 16 hours of refluxing is enough to complete the hydrolysis.

The hydrolyzed mixtures were allowed to cool, then the pH was gradually adjusted to pH 3 with 50-per cent solution of sodium hydroxide. As much as possible, the same amount of sodium hydroxide was used in the neutralization process by adding measured amounts of the alkali little by little to produce finished products of the same volume for all trials and to avoid the production of relatively dilute hydrolysates. They were then filtered to remove the solid particles. Subsequent to filtration, the hydrolysates were adjusted to pH 4.5 and filtered again through filter paper.

Mold enzyme digestion method.—This experiment also used five trials and utilized the same proportions of the paring meal to soybeans as given under the acid hydrolysis method. However, the paring meal and soybeans were soaked first in water overnight and autoclaved the next day at 15 pounds pressure for two hours, cooled and used as such as in the various trials.

The process described by Minor (8) was followed in the preparation of the enzyme hydrolysates. Fifty grams of each of the materials prepared for each trial were mixed thoroughly with equal amount of toasted wheat flour. Each mixture was inoculated with a mold culture of *Aspergillus oryzæ* propagated in the laboratory on potato dextrose agar. The inoculated trials were transferred into bamboo trays, covered with another bamboo tray and set aside for three days at room temperature. Durng this period, the inoculated mixtures were turned and broken up at least once daily. Uniform layers not thicker than 2 inches were maintained. The formation of heavy white mycelium throughout the mixture indicated that the mold had developed sufficiently. The mold mixtures were placed loosely in jars and agitated to eliminate large open spaces. A salt solution of 80° salometer reading was added just enough to cover each mass and fermentation was allowed to proceed for six weeks. The salt concentration was checked by means of the salometer from time to time and maintained at 80°. The mass was stirred once daily. After the sixth week the sauce was pressed out, filtered and pasteurized at 50°C for 30 minutes.

Each of the two above-mentioned methods of hydrolysis was conducted twice using the same batch of raw materials.

Examination of the hydrolysate condiments.—The hydrolysates were stored for six weeks at room temperature to age these products. Aging improves the organoleptic qualities of protein hydrolysate condiments. Representative samples of the five acid hydrolysates and the five mold-enzyme hydrolysates were analyzed for specific gravity, pH value, color, total titrable acidity, total solids, nitrogen and sodium chloride content and evaluated for flavor and aroma.

The methods followed in the various analyses are as follows: Specific gravity-Pycnometer method;(4) total solids — AOAC method;(1) sodium chloride — Open-Carius method;(1) nitrogen — modified Kjeldahl method;(5) total titrable acidity — method described by Onaga;(9) pH value — measured with the Beckman pH meter Model G; and color(9) — samples were diluted 100 times and color measured in a Klett Summerson photoelectric colorimeter using a blue filter No. 42 with spectral transmission ranging from 400 to 465 millimicrons.

Flavor and aroma.—The flavor and aroma of the samples were evaluated organoleptically by a panel of four members who are experienced in sensory evaluation. A randomized incomplete block design(2) was used in which the samples were served six times and each sample was served along with each other samples twice. At each session, the panel members were asked to rank the samples in the order of their preference for aroma and flavor. Samples were scored as follows: (*a*) good, (*b*) intermediate (c) poor. The results were analyzed statistically for least significant difference (LSD) and probability level of significance.(2)

RESULTS AND DISCUSSION

Chemical and physical properties of the acid and mold-enzyme hydrolysates are given in Tables 1 and 2. The values given are the average of two trials for each method used. The specific gravity of the sauces prepared by acid hydrolysis ranged from 1.1734

TABLE 1.—*Physical and chemical properties of acid and mold-enzyme hydrolysis.*

Trial	Specific gravity		Total solids		pH		NaCl, gm/100 ml		Nitrogen gm/100 gm		Titrable acidity per cent in terms of HCl	
	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme
1	1.1734	1.1497	27.12	21.3	4.2	4.3	17.94	20.37	0.35	0.35	1.29	1.21
2	[illegible]	1.1278	27.40	22.16	4.4	4.3	18.25	20.05	0.47	0.47	1.35	1.31
3	1.1738	1.1376	27.42	23.65	4.3	4.2	17.91	21.12	0.49	0.60	1.30	1.27
4	1.1741	1.1092	27.24	22.07	4.2	4.0	18.26	19.63	0.41	0.42	1.45	1.62
5	1.1716	1.1469	26.86	24.95	4.4	4.3	19.22	20.46	0.43	0.93	1.39	1.16

TABLE 2.—*Flavor, aroma and color of the acid and mold-enzyme hydrolysates.*

Trial	Mean flavor score*		Mean aroma score*		Optical density	
	Acid	Enzyme	Acid	Enzyme	Acid	Enzyme
1	2.3	2.04	2.8	1.92	0.12	0.004
2	1.6	1.88	1.8	1.92	0.11	0.005
3	1.5	2.00	1.2	2.04	0.11	0.012
4	2.2	2.96	2.8	2.9	0.12	0.017
5	2.7	1.88	2.8	2.08	0.12	0.009

* 1. best; 2. intermediate; 3. poor.

to 1.788 while that prepared by mold-enzyme digestion, from 1.1092 to 1.1409. For each type of sauce, specific gravity values did not differ much from one another. Acid and mold-enzyme hydrolysates prepared from soybean had almost the same specific gravity as those prepared from paring meal.

In general, the specific gravity of the acid hydrolysates was slightly higher than the mold-enzyme hydrolysates. Trial 3 of the acid hydrolysates prepared from a mixture of equal parts of paring meal and soybeans, gave the highest value for this physical property. As expected, it also exhibited the highest value for total solids content. Sauces with low specific gravity were correspondingly low in their total solids content. The total solids content contributes to the body of the sauce.

The pH values of the various trials after six weeks storage varied from 4.2 to 4.4 for the acid hydrolysates, and 4.2 to 4.3 for the enzyme hydrolysates. The initial pH of the acid hydrolysates was 4.5. It is at pH 4 to 5 that most amino acids and organic acids are present in the conjugated form or in the form of their salts. (10) These components give the distinct taste of the sauce.

The total titrable acidity of the acid hydrolysates ranged from 1.29 to 1.45 per cent and the enzyme hydrolysates from 1.16 to 1.68 per cent. For both types of hydrolysates, those prepared from a mixture of one part paring meal and two parts soybeans had slightly higher acidity values than the other hydrolysates.

Also given in Table 1 is the sodium chloride content of the various sauces which shows that the sodium chloride content of the enzyme hydrolysates were slightly higher than those of the acid hydrolysates. The latter contain from 17.94- to 19.28-per cent sodium chloride while the former from 19.63 to 20.46 per cent. Sodium chloride contributes to a great extent to the keeping quality of the sauce. Yokutsuka(12) believes that the sodium chloride should not be less than 16 per cent because of the danger of putrefaction. Since the sodium chloride content of all samples are above 16 per cent they can therefore be kept for long periods of time without danger of spoilage. It is a fact that salt content contributes to flavor acceptance of foods. In a study made by Onaga(9) on quality evaluation and chemical composition of soy sauce, results showed that optimum concentration of salt which may not impair the flavor of the sauce lies between 18 to 20 per cent. Except for Trial 3 of the mold-enzyme hydrolysates, the salt content of all the experimental sauces are within this range.

The amount of nitrogen in the acid hydrolysates ranged from 0.35 to 0.43 per cent while that of the enzyme hydrolysates, ranged from 0.35 to 0.93 per cent. For each type of hydrolysate, those prepared from paring meal alone gave the lowest nitrogen value while those prepared solely from soybean gave the highest.

Flavor and aroma scores and the color ratings of the five trials in both hydrolysates are shown in Table 2. The samples can be divided into three grades with respect to aroma and flavor. Trials 2 and 3 of the acid hydrolysates were ranked best in flavor and aroma. Between these two samples, there was no significant difference at a probability level of 5 per cent. Trial 2 was prepared from 2:1 mixture of paring meal and soybean while Trial 3, from a mixture containing equal parts of soybean and paring meal. Trial 1 (paring meal alone) and Trial 4 (paring meal and soybean at 1:2 ratio) were rated intermediate in quality in flavor but poor in aroma. Trial 5 prepared from soybean of the acid hydrolysate was the least liked by the panel in flavor and aroma.

Of the enzyme hydrolysates, those prepared in Trial 5 and in Trial 2 were judged to have the most acceptable flavor. However, their scores were slightly higher than those received by the acid hydrolysates (Trials 2 and 3) which were most preferred by the panel. Enzyme hydrolysates prepared from a mixture containing one part paring meal and 2 parts soybean (Trial

4) were rated poor in flavor and aroma while that prepared from a mixture containing equal amounts of soybean and paring meal (Trial 3) was ranked as of intermediate quality.

Optical density among the diluted acid hydrolysate samples did not vary much from each other. Trials 1, 4, and 5 had optical density of 0.22 while Trials 2 and 3, 0.11. In general, the acid hydrolysates were darker in color than the enzyme hydrolysates. The latter samples had optical density values ranging from 0.004 to 0.017 only. Color, just like flavor and aroma, is an important property of a sauce since it adds color to the cooked food. The color of locally manufactured sauces is generally derived from caramel added during the manufacturing process. The addition of a good quality caramel will improve the color of these sauces.

SUMMARY

Sauces were prepared by the acid and mold-enzyme hydrolysis methods using coconut paring meal, soybeans and combinations of paring meal and soybeans in varying proportions. The various sauces were analyzed for pH, acidity, sodium chloride, specific gravity, nitrogen and total solids. Flavor and aroma were evaluated using the randomized incomplete block design and color was measured in a Klett-Summerson colorimeter. In general, the acid hydrolysates gave higher specific gravity and more total solids than the mold-enzyme hydrolysates. For all samples, pH, acidity and sodium chloride content varied slightly from one another. The acid hydrolysates exhibited higher optical densities, indicating that their color is darker than those of the mold-enzyme hydrolysates. Hydrolysates prepared solely from soybeans had the highest nitrogen content.

Results of organoleptic tests showed that acid hydrolysates prepared from mixtures containing two parts of paring meal and one part soybean and from equal parts paring meal and soybean were most preferred in flavor and aroma by the panel. The sauces rated least were the acid hydrolysates prepared from soybeans alone and the enzyme hydrolysates prepared from a mixture containing one part paring meal and two parts soybean meal. However, enzyme hydrolysate prepared from a 2:1 mixture of paring meal and soybean was most preferred in both flavor and aroma by the panel.

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BARK AND CAMBIUM REGENERATION AS A FACTOR IN PLANT AIR-LAYERING

By LEOPOLDO B. UICHANCO [1]

(Received for publication, March 8, 1968.)

The art of asexual plant propagation has been developed mainly in countries of the North Temperate Zone, where the method in its various forms has been in common use to insure true-to-type multiplication of desirable varieties and strains or to multiply varieties or strains which do not produce seeds. Of the numerous techniques available, air-layering, which in the Philippines is usually called "marcotting," has been much favored over such alternatives as budding and grafting, because it demands of the operator no special surgical skill and moreover, no problem of stock-scion incompatibility is encountered. This popularity subsists, despite the fact that air-layering takes as long as some two-and-a-half to four months, depending on the plant material and the prevailing weather, before the treated branch can grow enough roots of its own to permit being weaned from the parent.

Like many other practices originating in the temperate countries, air-layering is not infrequently attended in the tropics with some vexing problems. On certain plant varieties, callus and root formation is a painfully slow process, if it does not fail altogether. One notably perverse case was met in a rather uncommon yellow-orange variety of Ixora (santan). A magnificent specimen stood out because of its prodigally colorful, year-round flower in the writer's yard at U.P. Village, Quezon City.[2] This prize acquisition had been presented to Mrs. Uichanco some four years ago, by her late friend, Mrs. Fernando Ordoveza, out of a rich plant collection gracing the Ordoveza estate at Bay, Laguna. The gift consisted of a large flower pot bearing a particularly lusty specimen of air-layered Ixora. Although still small the plant was even then in haste to bare its florid charm.

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[2] On November 3, 1967, Typhoon "Welming," the Philippine Weather Bureau name for it, struck Greater Manila and battered this bush fiercely, leaving it in shambles. However, at this writing (November 27), it is fast rising up from the ruins with its vigorous new shoot and a fresh crop of flower clusters.

In our former home on the campus of the College of Agriculture, as well as in its new location in Quezon City, this exotic beauty proved to be quite shy resisting all overtures proffered towards its multiplication, i.e., by cutting, grafting or air-layering. On closer examination of the air-layered branches, it was found that this variety, instead of producing callus and roots, vents its energy into regenerating the cambium and bark tissues. This led the writer to try a modification of the traditional air-layering method, which may be new or little known. Partway on the wood exposed by bark-ringing, a couple of turns of No. 20 galvanized wire were wound around the stem, leaving the two ends close together and protruding a few millimeters to give the pliers a good grip as these are twisted into a short braid. Care was taken neither to tighten the wire too much nor leave it too loose.

As rooting medium, a well-moistened mixture of equal parts of bird's nest fern (*Asplenium nidus* L.) root fiber and garden soil was found most suitable. The ideal material for cover proved to be polyethylene plastic sheets, such as the emptied bread loaf wrapper. It is suitable for the purpose since it was neither so thick that it became unmanageable nor so thin as to break easily during use. A piece of the plastic sheet of convenient size was wrapped around the rooting medium on the girdled portion of the stem and tied firmly on the two ends and in the middle to keep it from slipping down to the base or turning around; otherwise the tender new roots might be ruined.

The modified air-layering method was first used on six branches that had been bark-ringed and covered four months before, but did not produce any root. They were ringed again on August 28, 1967 and then wire-choked by the above technique. Each of the branches was found to disport a good growth of roots about one-and-a-half months after this retreatment. Subsequent trials on originally virgin branches elicited roots in about two months.

The new technique was also tried on a Citrus plant which was suspiciously a natural hybrid of lime X Calamondin, with equally gratifying results. However, that particular Citrus form eventually proved to be too accommodating; even the trimmings pruned from the rooted branches could be grown easily. Girdling with wire as described above was also tried on roses, but in this case without bark-ringing and without wrapping in rooting media. Limited trials showed that in four to six weeks, the wired sections could be cut and grown much more successfully than the fresh unconditioned branches. With unconditioned cuttings, failure in some varieties was more the rule than the exception.

The question may well be asked: Why should not the simpler standard method of air-layering work just as well in the tropics as it does elsewhere? The precise answer is probably not to be found in the present state of our knowledge. However, although the probability of inherent constitutional differences between tropical and temperate plant forms cannot be ruled out, general observation seems to point to environmental factors as the probable root cause. The uniformly high temperature and damp air prevailing the year round in the humid tropics, as in the Philippines, make for lush vegetative growth among terrestrial plants, as compared with the normally dry air and numbing cold a good part of the year in the temperate climate. For example in particularly wet Philippine localities, such as Lilio and Majayjay in Laguna Province and Surigao, the fiber-thatched roofs are thick-carpeted with moss, with papaya and other plant seedlings sprouting helter-skelter from seeds carried thereto by wind or with the droppings of wild birds and civets. The tree boughs are thick with long fringes of the common epiphytic fern, *Pteropsis pselloselloides* (Linnaeus) Desvaux. This situation, in contrast, is not found in any temperate locality.

One striking instance of differential response of plants to climate is afforded by the following incident. On a visit to Harvard's Arnold Arboretum in Forest Hills, off Boston, Massachussetts, some twelve years ago, the writer was shown striking examples of dwarf apples which Dr. Karl Sax, its former director, had produced by a novel method that he had described some months previously in *Science*. The miniature apple trees (or shrubs), although of greatly reduced dimensions, actually bore more fruits and oftener, according to Doctor Sax. His method was simplicity itself; it consisted in excising the bark around the sapling's trunk, as near the base as possible, into a ring band of uniform width. The bark forming the band was kept intact, except where the side cut is made for detachment. Once removed, the band is restored to its original place, but in an upside down position. The inverted ring, if it takes, then grows as an integral part of the entire trunk's bark, but, as a result of the operation, dwarfing ensues. This intriguing demonstration conjured visions of exciting possibilities, among them a garden plot back home laden with fruits and flowers in infinite variety as these are borne in rich profusion on rows upon rows of miniaturized trees.

When the writer returned to his station, which was then in Los Baños, he lost no time and set about busying himself as he tried to duplicate the Sax experiments, but on Philippine plant material. Very many sapling's were needlessly mutilated, now avocado, now jakfruit, now mango, now Bougainvilla, but all with no success. In each case the bark in the band collapsed into any number of fragments during the operation. Finally seedlings of the santol, *Sandoricum koetjape* (Burm. f.) Merrill, turned out to be more cooperative, so a good assemblage of potted seedlings of this tree, each meekly nursing a live and healthy inverted bark band near the base of the trunk, was soon at hand. The reward for all the long and arduous toil came about after several months of hopeful expectation, which unhappily spelled the end of the writer's tree-dwarfing dreams. Every single one of the tampered seedlings sprouted a luxuriant fringe of lusty new roots bordering the upper edge of the inverted bark band, which was merrily by-passed forwith as the roots reached down and established direct communication with the soil. The surgical operation was successful, but to the patient it was merely a mild annoyance, when the seedling unconcernedly resumed its normal life and once more proceeded to develop like a good santol should.

It could not be that the Philippine santol is more resourceful than the American apple. Here again, high tropical humidity in combination with consistently high temperature have most probably conspired to foster our santol's wilful disposition and save it from the ignominy of a freak's life. Its tender aboveground roots might have shrivelled up and died aborning had the same santol seedling been exposed instead to the dry air of the temperate latitudes.

EFFICACY OF NATIVE TAN BARKS AND CHEMICALS USED IN THE PRESERVATION OF COTTON TWINES USED IN THE CONSTRUCTION OF FISHING NETS

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SIX TEXT FIGURES

The use of vegetable fibers in the construction of fishing nets is still very popular in the Philippines. In some developed countries like the United States of America, Japan, England, etc., they have changed from natural to man-made fibers. Maintenance of fishing nets has to be on a sound economic level to create substantial profits for the benefit of the industry.

The main objective of this research investigation is to determine methods which would prove most suitable for the preservation of nets and twines under local climatic and operative conditions.

Untreated vegetable fibers on prolonged exposure to water deteriorates rapidly especially in tropical countries. In the Philippines where there is intense sunlight and high temperature, bacterial attack on the cellulose content is fast. The rotting activity can at least be minimized or prevented with the use of suitable rot resistant preservatives.

The usual practice of local fisherman is to wash the net thoroughly in water and dry very well after use. For many years, they have adopted such measures as preserving their nets with tanning extracts, animal blood, albumin, etc. Their methods are quite laborious and time consuming; besides, washing and drying of nets under the sun is impractical during rainy season and also unsuitable to gears on prolong immersion like trap and set-nets. The following series of tests and experiments utilized organic and chemical preservatives.

REVIEW OF LITERATURE

Coal tar is a common net preservative used in many countries. Various authors, however, have different opinions about the methods of application of coal tar as a preservative for cotton twines. Firth and Carlson (1947) recommend coal tar as an effective preservative and also suggest that it should be heated up to a temperature of 65° to 80°C (150° to 180°F) to insure better penetration. Whiteleather and Brown (1945) considers that coal tar should be avoided particularly in the tropics. Carothers (1949) also recommends the preservation of cotton web with coal tar. Sulit and Panganiban (1954) are of the opinion that coal tar treatment is effective particularly when resin is admixed in small quantities. Tokayama and Shimozaki (1957) also favor the view that this treatment shows more preservability of the fiber.

Tannin in its pure state is highly soluble or leachable in water. The tannin content can be fixed with the aid of a fixative as potassium dichromate ($K_2Cr_2O_7$), copper sulphate with liquor of ammonia and sodium dichromate [Miyamoto (1958)]. Migita (1943) used 1 per cent of potassium dichromate solution to effect superior tanning, while Brandt (personal communication) suggests 3-per cent solution of the same oxidizing agent for tannin fixation.

MATERIALS AND METHODS

a. *Organic preservatives.*—The sources of organic preservatives commercially used in the Philippines are tannin extracts from bark of trees, animal blood and albumin.

The tanning fixation used in this study is approximately 3 per cent of potassium dichromate. The dried twines were dipped in a decoction of 6 kilos tan barks (nigi — *Xylocarpus* sp. and tangal — *Ceriops roxbhurgiana*) in 5 gallons of water for 3 times. Ample time for drying is given before a re-dipping it in the same solution. The dried tanned twines are dipped in the solution of potassium dichromate for 10 to 15 minutes after which they are washed in plain water to remove the excess potassium dichromate.

b. *Chemical preservatives.*—The chemical preservatives utilized in this study are coal tar and potassium dichromate. Coal tar is a common chemical preservative used in different countries including the Philippines.

Experiments conducted at Dagat-dagatan research laboratory utilized coal tar thinned with gasoline and coal tar on tanned twines.

c. *Cotton twines.*—Cotton twines Nos. 20/6, 20/9, 10/6, 10/9, 10/12 and 10/15, all medium laid, were utilized to determine the efficacies of preservatives, and all were locally manufactured by Everbright Development Co. (Camia Brand).

d. *Apparatus.*—A yarn tensile strength tester "Shimadazu" brand manufactured in Japan was used in testing the strength of the twines at various treatments and exposures. This apparatus is electrically motor driven, 1/4 H.P. (0.2KW), 100V, A.C., single phase, for quick and accurate testing of every sort of twisted yarn. Through mediation of a speed reducing device, power from the motor is transmitted to a lower clamp to give it a downward motion which exerts a load on a sample. Raising and lowering of the clamp is performed by means of a lever.

Capacity in 2 ranges: 0—20 kg in 100 gm and 0—10 kg in 50 gm.

Test length:	200—500 mm
Size of tester:	75 × 46 × 196 mm
Net weight:	95 kg.

EXPERIMENTAL PROCEDURE

The cotton twines were cut into equal length of 30 inches and prepared into bunches. Each bunch contained 75 pieces. The prepared twines were strung in hang lines and dipped in their corresponding preservatives.

a. *Tanning with tan bark extracts using tangal, nigi, with potassium dichromate.*—The cotton twines prepared above were soaked in boiling water for 5 to 10 minutes to remove wax or any foreign particles adhering to them. All the twines were similarly treated before they were actually dipped in the various preservatives mentioned below. The solution of tan barks was prepared by boiling 6 kilos of tan barks (3 kg. tangal plus 3 kg. nigi) in 5 gallons of water. The solution was boiled and concentrated to $\frac{1}{4}$ of the original volume. The cotton twines were then dipped in the hot solution for three times after which they were dried. Two hundred-fifty grams of potassium dichromate was dissolved in 3 gallons of water and boiled. The tanned cotton twines were dipped in the boiling solution for 10 to 15 minutes after which the treated twines were dried under the shade.

b. *Tan bark extracts and animal blood.*—The solution of tan barks was prepared by boiling 6 kilos of tan barks (3 kg. tangal plus 3 kg. of nigi) in 5 gallons of water. The solution was boiled and concentrated to ½ of the original volume. The cotton twines were then dipped in the hot solution for three times after which they were dried.

The tanned twines were next dipped in animal blood until uniformly coated (blood was purchased from Manila slaughter house). Then the treated twines were steamed for 3 hours and dried under the sun.

c. *Albumen.*—The twines were thoroughly dried, then dipped in a mixture of 1 part albumen and 6 parts water until uniformly coated. They were next dried under the sun, then steamed for 2 hours. Finally, the twines were dried under the sun.

d. *Coal tar thinned with gasoline.*—A mixture of 1 part of gasoline to 3 parts coal tar (Master brand) was prepared and stirred to a homogenous mixture, without the application of heat. The twines were then dipped for 15 minutes until no bubbles appeared at the surface. The excess tar was allowed to drip from the twines, then dried in the shade.

e. *Coal tar thinned with gasoline and tan bark extracts from nigi and tangal.*—The tan bark solutions were prepared by boiling 6 kilos of tan barks (3 kg. tangal plus 3 kg. nigi) in 5 gallons of water. The solution was boiled and concentrated to ½ of the original volume. The cotton twines were then dipped in hot solution for three times after which they were dried.

A homogenous mixture of 1 part gasoline to 3 parts coal tar (Master brand, factory prepared) was made without the application of heat. The mixture was stirred and the tanned twines were dipped in it for 15 minutes until no bubbles appeared at the surface. The excess tar was allowed to drip from the twines after which the twines were dried in the shade.

f. *Pine tar application without heat.*—The twines were dried under the sun and dipped in factory prepared pine tar. The excess pine tar was allowed to drip from the twines and hanged in the shade to dry.

The treated twines were subjected to three types of exposures, namely:

1. Weathering — treated twines were exposed to atmospheric conditions and sampling was done every seventh day.
2. Continuous immersion in brackish water — the twines were continuously immersed in brackish water until the zero tensile strength was reached. Sampling was done every seventh day.

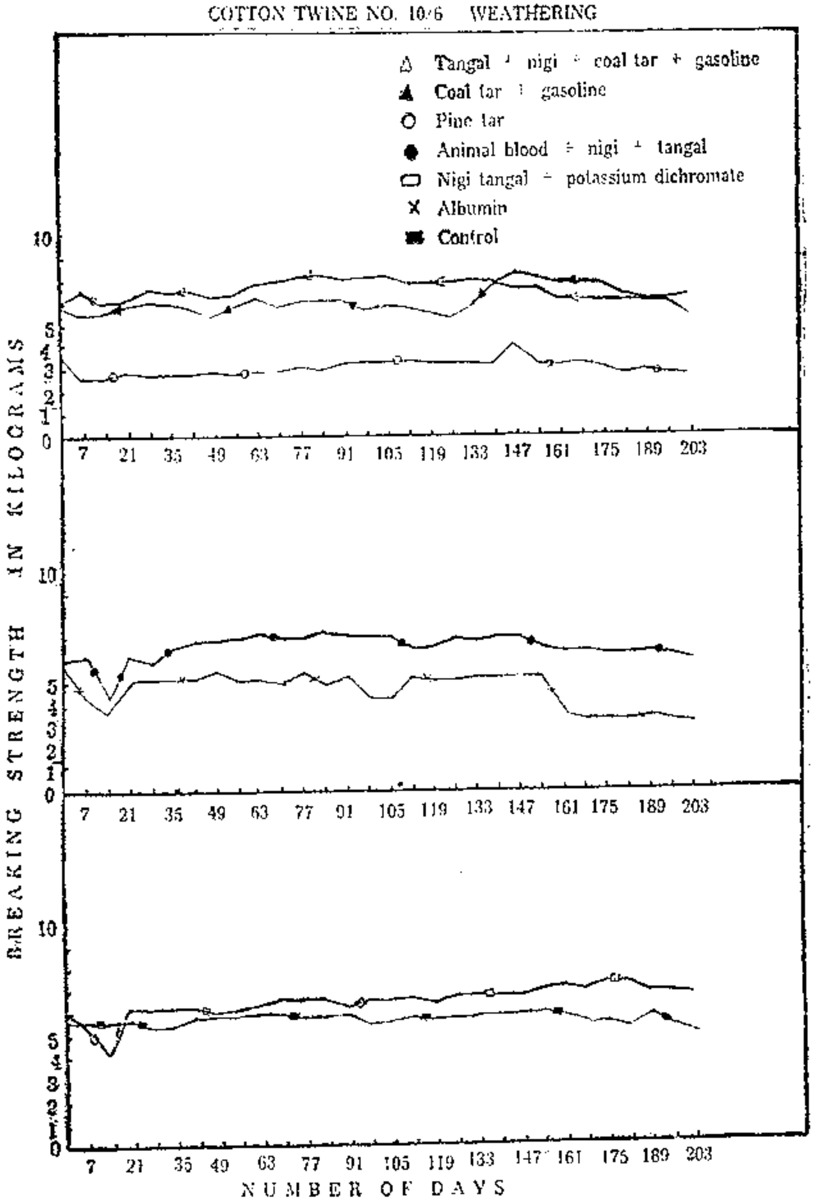

FIG. 1. Comparative deterioration rate in treatment weathering.

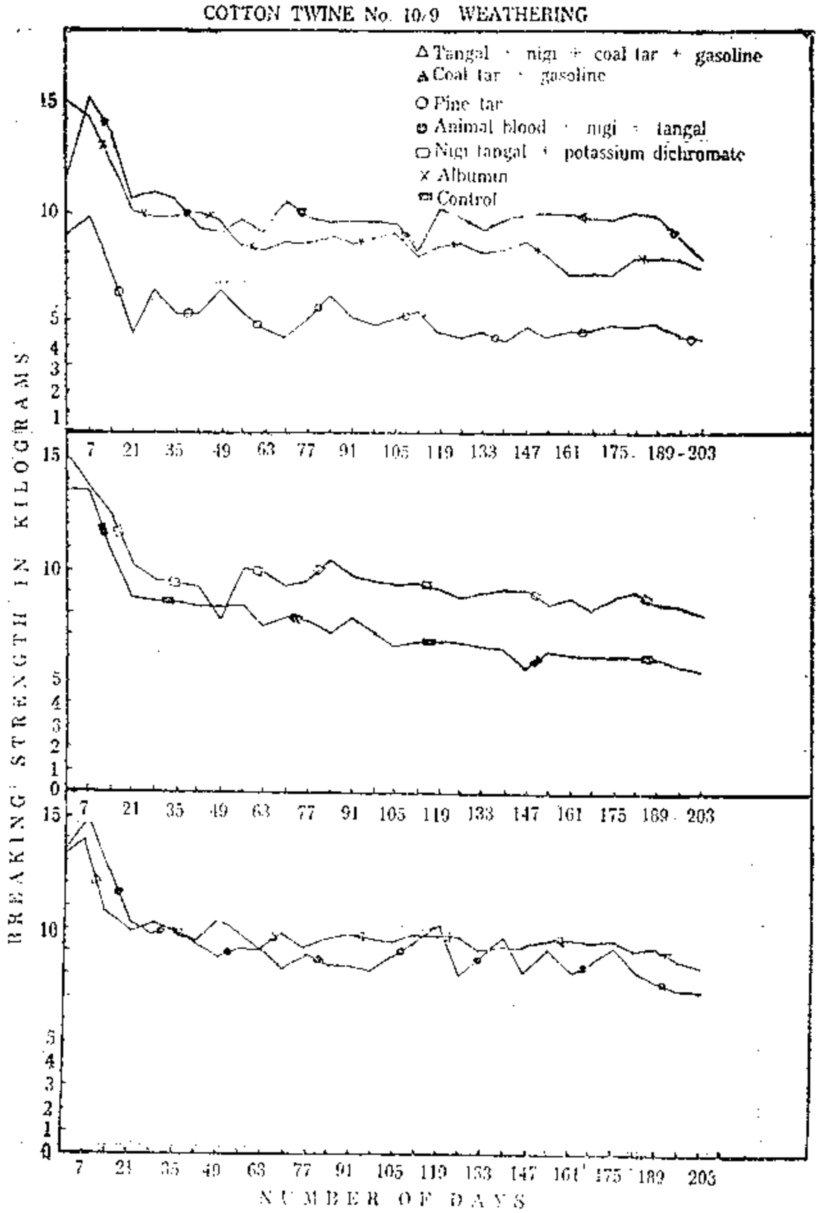

FIG. 2. Comparative deterioration rate in weathering treatment.

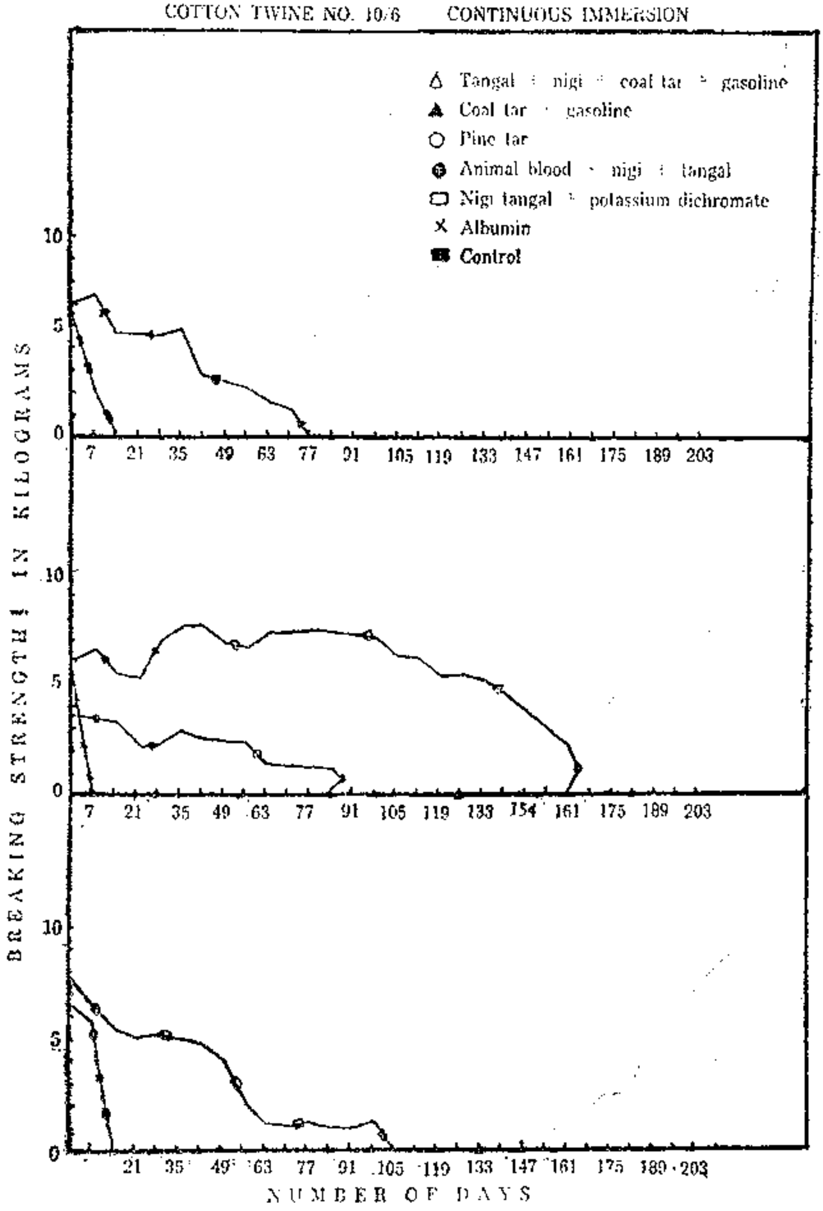

FIG. 3. Comparative deterioration rate in continuous immersion treatment.

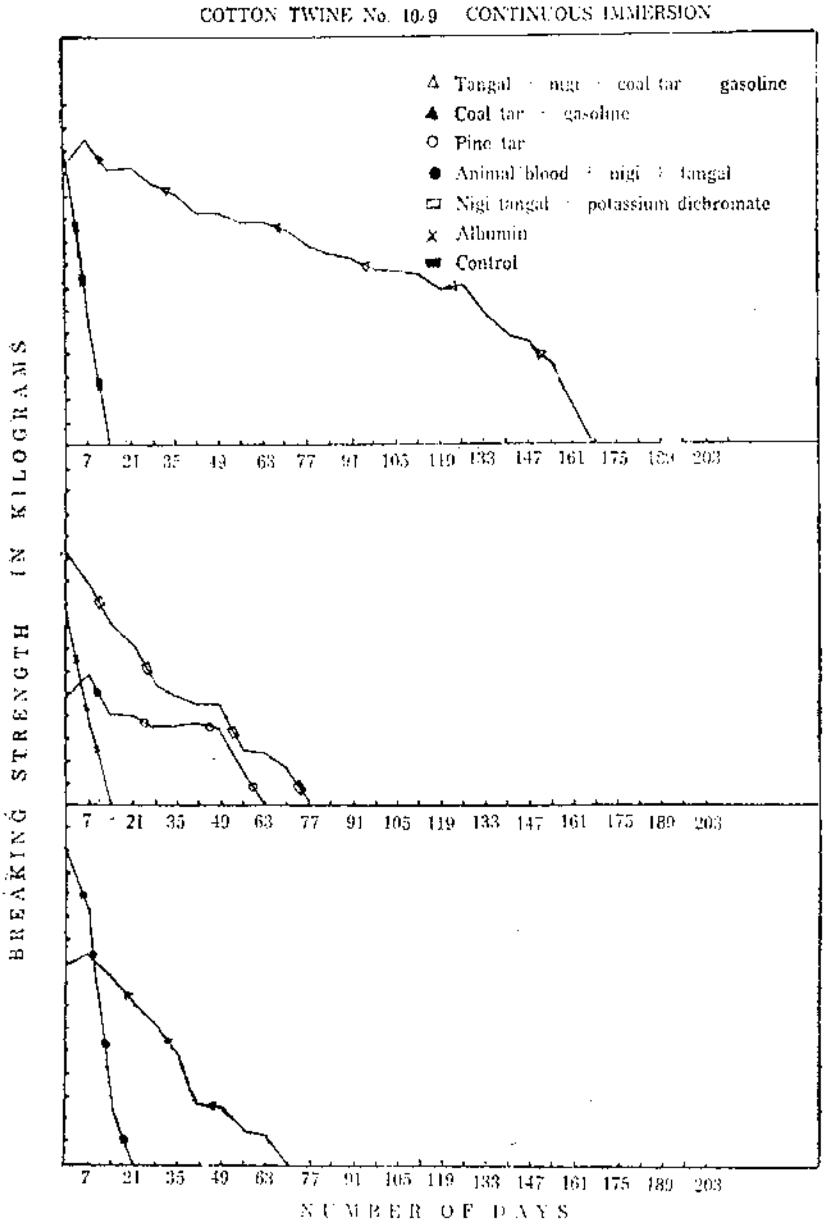

FIG. 4. Comparative deterioration rate in continuous immersion treatment.

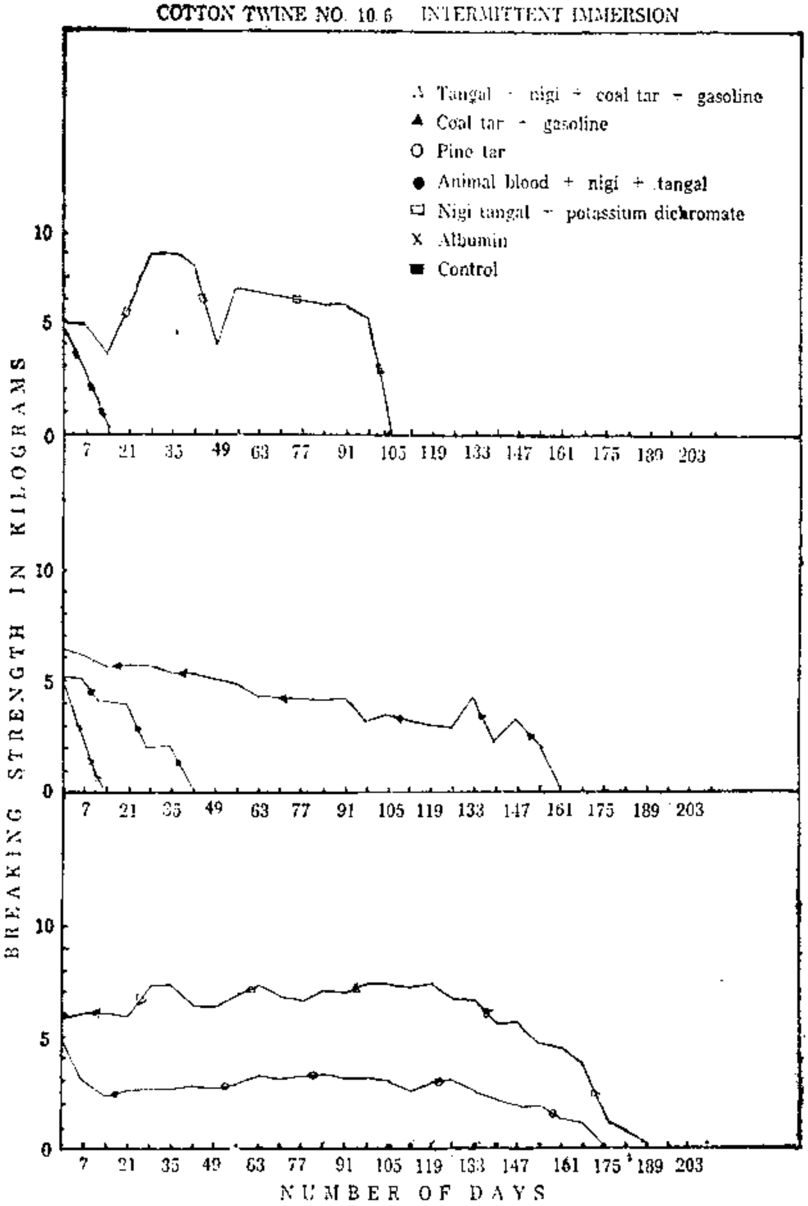

FIG. 5. Comparative deterioration rate in intermittent immersion treatment.

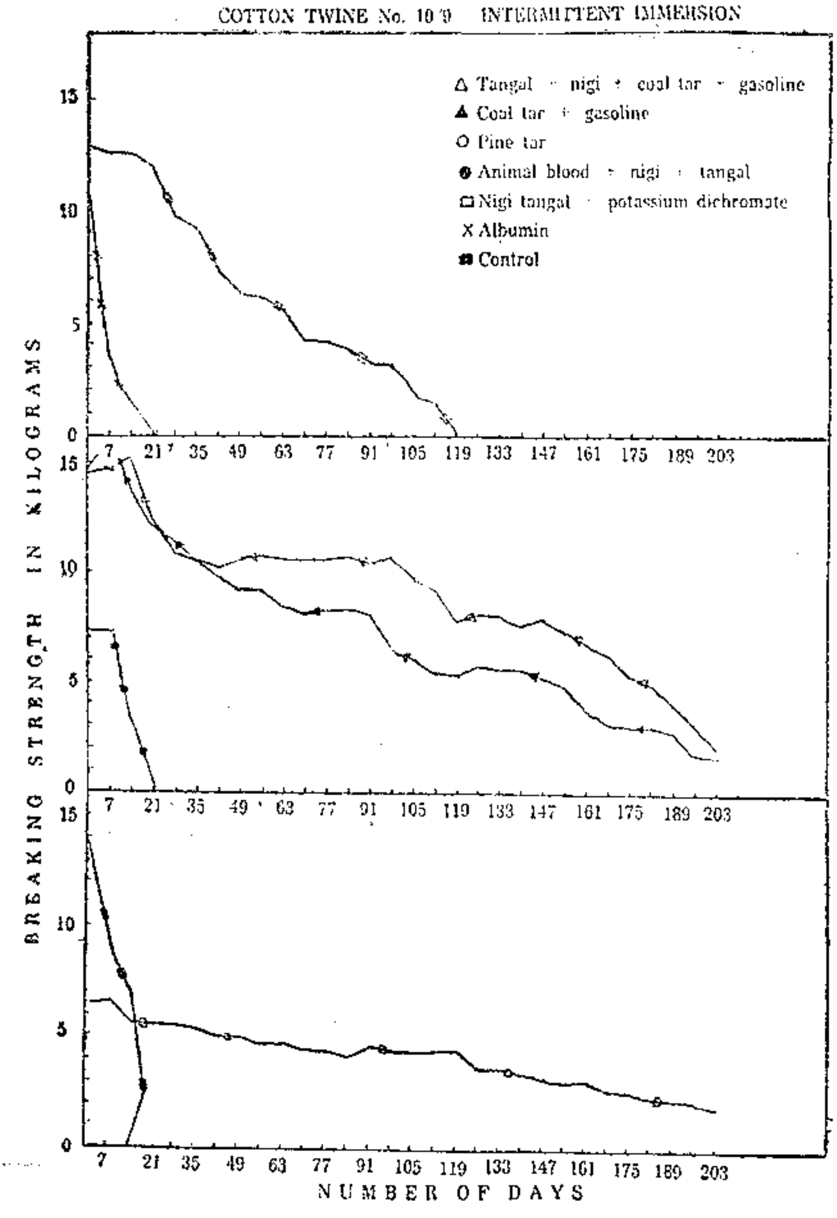

FIG. 6. Comparative deterioration rate in intermittent immersion treatment.

3. Intermittent immersion in brackish water — the twines were immersed on the first and second days, removed on the third day and dried on the fourth day. They were re-immersed on the fifth and sixth days and removed and sampled on the seventh day.

This procedure was followed every week throughout the whole duration of the experiment. Sampling of water for salinity determination and twines for tensile strength tests were performed every seventh day.

Tests for breaking strength of each of the set of twines were done periodically to determine the relative breaking strength of the twines at various treatments.

Temperature and salinity determinations in the experimental site were taken during the period of the experiment. The average monthly readings are shown in Table 3.

RESULTS AND DISCUSSION

Table 1 shows that the experimental twines treated with decoction of tan barks and coal tar thinned with gasoline is the most effective treatment as indicated by the slow rate of rotting activity in various types of exposure; namely, weathering, continuous, and intermittent immersions.

Table 2 indicates the percentage of impregnation of the various preservatives used in the experiment. Tan bark, and coal tar diluted with gasoline showed the highest percentage of impregnation followed by pine tar and coal tar diluted with gasoline. Albumen had the least percentage of impregnation.

In the intermittent immersion treatment of various twines (10/6, 10/9, 10/12, 10/15, 20/6, and 20/9), the combination of tangal, nigi, coal tar and gasoline showed good properties as a preservative followed by pine tar and coal tar diluted with gasoline. The tan bark and animal blood and albumen treatments gave very poor results in immersion tests.

In the weathering exposure groups all the treated twines still registered breaking strength after 203 days of exposure to weather elements, except for size 20/6 which still registered after 175 days, size 10/12 (No. 6) after 189 days and size 10/12 (No. 7), 196 days. Here the rate of deterioration was gradual which was unlike the other exposures. One reason may be attributed to sunlight disinfection which lessens bacterial action on the cellulose content of the fiber.

TABLE 1.—*Relative breaking strength of the various experimental cotton twines after certain periods of treatment and exposures.*

Treatment	Sizes of experimental cotton twines											
	10/9		10/6		20/6		10/15		10/12		20/9	
	Breaking strength in kilograms	Rots after (in days)	Breaking strength in kilograms	Rots after (in days)	Breaking strength in kilograms	Rots after (in days)	Breaking strength in kilograms	Rots after (in days)	Breaking strength in kilograms	Rots after (in days)	Breaking strength in kilograms	Rots after (in days)
A. Continuously submerged:											0	161
1. Tangal, nigi, coal tar, and gasoline	0	167	0	168	0	119	0	168	0	168		
2. Nigi, tangal, and potassium dichromate ..	0	77	0	105	0	49	0	98	0	91	0	63
3. Coal tar and gasoline ...	0	70	0	77	0	42	0	77	0	91	0	42
4. Pine tar	0	63	0	91	0	35	0	98	0	72	0	35
5. Animal blood, nigi, and tangal	0	21	0	14	0	14	0	21	0	21	0	14
6. Albumen	0	14	0	7	0	7	0	14	0	14	0	7
7. Control (untreated)	0	14	0	14	0	7	0	14	0	14	0	7
B. Intermittently submerged:												
1. Tangal, nigi, coal tar, and gasoline	2.18	Tensile strength still registered after 203 days	0	189	0	126	0	203	7.0	Tensile strength still registered after 203 days	0	196
2. Pine tar	2.06		0	175	0	105	0	189	3.2 0 0		0	147
3. Coal tar and gasoline	1.7		0	161	0	48	0	196	0	203	0	140
4. Nigi, tangal, and potassium dichromate ..	0	119	0	105	0	56	0	105	0	98	0	84
5. Animal blood, tangal, and nigi	0	21	0	42	0	35	0	56	0	56	0	35
6. Control (untreated)	0	14	0	14	0	7	0	14	0	14	0	7
7. Albumen	0	21	0	14	0	14	0	14	0	14	0	7
C. Weathering:												
1. Coal tar and gasoline	7.7		5.2		2.9		15.3	Tensile strength still registered after 203 days	14.2	Tensile strength still registered after 203 days	3.7	Tensile strength still registered after 203 days
2. Tangal, nigi, coal tar, and gasoline	8.3	Tensile strength still registered after 203	6.1	Tensile strength still registered after 203	3.0	Tensile strength still registered after 175	16.5		14.5		4.2	
3. Animal blood, nigi, and tangal	7.4		5.6		2.2		13.3		13.0		3.5	
4. Nigi, tangal, and potassium dichromate ..	8.2		5.6		2.6		15.6		12.5		3.5	
5. Control (untreated)	5.7		3.9		1.8		11.8		8.4		3.0	
6. Albumen	7.32		2.9		2.2		12.0		11.7	Tensile strength still registered after 189 days	3.0	
7. Pine tar	4.1		2.7		1.6		6.2		6.0	Tensile strength still registered after 196 days	1.8	

TABLE 2.—*Percentage of impregnation of various preservatives.*

Preservatives	Sizes of experimental cotton twines																	
	20/6			20/9			10/6			10/9			10/12			10/15		
	weight in grams			weight in grams			weight in grams			weight in grams			weight in grams			weight in grams		
	After treatment	Difference in weight	Per cent of impregnation	After treatment	Difference in weight	Per cent of impregnation	After treatment	Difference in weight	Per cent of impregnation	After treatment	Difference in weight	Per cent of impregnation	After treatment	Difference in weight	Per cent of impregnation	After treatment	Difference in weight	Per cent of impregnation
a. Tan bark and coal tar diluted with gasoline	31.5	18.9	150	43.3	25.3	142.72	72.6	43.0	145.26	108.4	58.0	115.07	143.8	77.9	118.2	186.1	101.8	120.75
b. Coal tar diluted with gasoline	28.8	15.8	121.53	39.6	20.6	108.42	58.4	28.3	94.02	95.2	45.2	90.4	125.6	60.1	91.75	162.3	77.3	90.94
c. Pine tar	28.0	15.4	121.22	38.9	20.17	113.41	68.0	38.4	129.72	102.0	51.5	101.98	130.0	63.2	94.61	177.3	92.3	109.88
d. Animal blood and tan bark	14.5	1.4	13.28	20.0	11.5	8.1	31.9	3.3	11.53	53.7	3.1	6.12	73.8	7.8	11.81	90.4	5.8	6.85
e. Tan bark and potassium dichromate	14.4	1.5	11.62	19.9	1.7	9.34	31.7	2.1	7.09	52.0	1.5	3.0	70.0	3.2	4.79	82.4	1.6	5.0
f. Albumen	13.3	0.5	3.9	19.72	0.7	3.78	30.4	1.4	4.84	51.8	1.2	2.37	67.8	1.8	2.72	85.2	0.6	1.89

TABLE 3.—*Average temperature and salinity.*

Month	Average temperature		Salinity (o/oo)
	Air (O°C)	Water (O°C)	
September, 1962	29.0	30.1	10.94
October, 1962	29.7	30.15	16.77
November, 1962	28.92	28.58	26.57
December, 1962	27.5	26.65	24.47
January, 1963	25.97	25.82	23.69
February, 1963	25.6	25.1	31.09
March, 1963	25.10	26.2	31.25
April, 1963	27.45	27.75	32.23

Efficacy of preservatives.—The combination of tan barks and potassium dichromate showed indications of a good preservatives. The tannin content is efficiently fixed by potassium dichromate. However, precautions should be taken with its use as the vapors when inhaled are dangerous to health.

The combinations of tan barks and animal blood and albumen are not very effective as shown by the results. However, they may give protection to twines against abrasions by forming a coating on the surface. Nets preserved with these combinations will last longer if proper sunlight disinfection and through washing in fresh water are given to nets.

In the experiment where a mixture of coal tar and gasoline was used, the efficacy was good but limited because gasoline acted as dryer only. When coal tar thinned with gasoline was applied on tanned twines, the efficacy was greater than the coal tar-gasoline combination because coal tar acted as a fixing agent.

In the case of pine tar, the problem of drying was encountered. It may give good indications as a preservative but it is not usually applied by fishermen, because it is cumbersome to handle, sticky and slow to dry. Normally it takes several days to dry making it economically unwise to use. Some kind of drying agent or heat treatment may solve the problem.

CONCLUSION

Based on the results of the experiment, the combination of tan bark and coal tar diluted with gasoline appeared to be satisfactory as shown by the results of the tensile strength tests. Under this three types of exposure conditions, this combination showed very good results as compared with the other combinations. Further improvement may still be attained by the use of a good fixative. However, coal tar diluted with gasoline showed also good results. The decoction of tan bark fixed by potassium dichromate is also an effective preservative especially if the nets are retreated occasionally. Animal blood and tan barks and albumen are effective preservatives if the nets are given proper sunlight disinfection and thorough washing in fresh water. This is true only in nets which are used periodically.

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PROTEIN FROM SEAWEEDS FOR ANIMAL FEED SUBSTITUTES

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THREE TEXT FIGURES

Interest has been focused lately in the Philippines on the utilization of seaweeds for human and animal feed, because of their nutritive qualities, digestibility and potentialities for growth. Wide seaweed areas surround the Archipelago. Seaweeds as animal feed have not been very well appreciated because of inadequate information regarding their food value. However, the Japanese and Chinese utilize seaweed not only for its medicinal value but more particularly as a food delicacy. In Great Britain and Scandinavian countries, it is extensively used as animal feed.

This project aims to determine the species of Philippine seaweeds and algæ that could be used as a substitute for fish meal in the preparation of animal feed. Being a cheap source of food material, the incorporation of seaweeds and algæ would lower the cost of animal feed production and decrease the annual fish meal importation from foreign countries. Through this project information provided can help develop a stable seaweed industry which can be of benefit to our local poultry, piggery, and livestock industries.

REVIEW OF LITERATURE

The protein of seaweeds, as in other plant proteins is less assimilable than the animal protein. Black, in 1953 found that "if brown algæ is fed only to the extent of 10 to 20 per cent of the diet, the protein contribution is negligible." The chemical composition of seaweeds and manufactured products vary according to the source of seaweed samples, season of the year when collected, and to the parts of the plant utilized. According to Sanford (1956) the major constituents include protein, fat, minerals and vitamins. Coulson in 1953 worked on hydrolyzates of algal protein and found them to contain essential amino acids. Lundes in 1939, found in his experiments with pigs, rats, horses, and poultry that an addition of 5- to 10-per cent seaweed to the ration was very beneficial.

EXPERIMENTAL PROCEDURE

The seaweed sample was analyzed in the chemistry laboratory for its protein, mineral, and moisture content. The percentage of protein in the sample was calculated on the total nitrogen using 6.25 as the conversion factor. Standard laboratory procedures were followed in the preparation of experimental diets for growth study.

The standard experimental diet requires 12 to 15 per cent of protein in the seaweed and fish meal samples. To determine the percentage protein in seaweed to be incorporated with fish meal, 8 per cent of the total protein content of seaweed and 4 per cent of the total protein content of fish meal were taken to attain 12-per cent protein in the experimental diet.

The rats utilized in the experiment were from 28 to 30 days old. Each group had equal number of males and females from the same litter. Individual rats had similar weights and initial group weights were about the same.

Rats were divided into 18 groups of 8 according to the experimental diets under investigation. The rats were placed individually in separate cages with raised screen bottoms provided with paper towels to prevent the loss of spilled food. A certain weighed amount of the diet was given in the feeding experiments, while food and water were given *ad libitum.* The food intake of each animal was carefully recorded for the six-week period of study.

Weights of the experimental animals were recorded every other day. Observations on the health or any abnormal development and physiological manifestations of the rats were made daily.

RESULTS AND DISCUSSION

Seaweeds and fish meal constituted the major source of protein in the diets of the experimental rats. To determine the biological potencies of the seaweeds under investigation, comparisons were made of the growth rates of the various groups fed with different proportions of seaweeds and fish meal. *Galongong* fish meal was used because of the availability of galongong in local markets throughout the Philippines. Commercially, it is a cheap fish yet its protein content is relatively high.

Hypnea was incorporated in three experimental proportions and gave the highest rate of growth followed by *Asparagopsis.* Under this condition of the experiment, seaweeds can be utilized with some food of high nutritive value as protein supplements for animal feeds.

In Fig. 3, the feeding test on rats showed that the protein of the brown algæ under investigation had low biological value. In any case the authors believe that if brown algæ can be fed only to the extent of 10 to 20 per cent of the diet [Black (1953)], the protein contribution is negligible.

Sargassum which belongs to the group of brown algæ gave almost the same result as *Hydroclathrus cancellatus,* their protein contents being 9.70 and 9.28 per cent, respectively, as shown in the bio-assay analysis.

Results reveal that the groups which were fed with the *Enteromorpha* and *Cladophora* diets did not show any increase in weight. Consequently, they appeared unhealthy and inactive with no interest in things around them.

The average growth of the rats fed with pure seaweed diet was very much slower than the growth of rats fed with seaweeds and fish meal (Fig. 1).

The bio-assay experiments indicate that, with a protein content of 11.83 per cent, *Asparagopsis taxiformis* (bulaklak bato) can support growth at the proportion of 4-per cent seaweed to 8-per cent fish meal. Results indicate that the seaweed contains some essential amino acids necessary for growth. In view of its abundance and availability in Philippine waters it has good prospects as a protein source for animal feeds. It will help attain one of the primary objectives of the Philippine Fisheries Commission — the decrease of our yearly importation of fish meal.

In the case of *Enteromorpha intestinales* (bitukang manok) and *Cladophora* (lumut jusi), experimental results show that, inspite of their high protein content (*Enteromorpha,* 17.82 per cent and *Cladophora,* 19.02 per cent), their effect on the growth of rats was not satisfactory at levels of 8-per cent seaweed meal and 4-per cent fish meal.

Combination of equal proportion of *Enteromorpha, Cladophora,* and fish meal was tried and the effect on the growth of rats was better than the proportion mentioned above but was still way below that of the control.

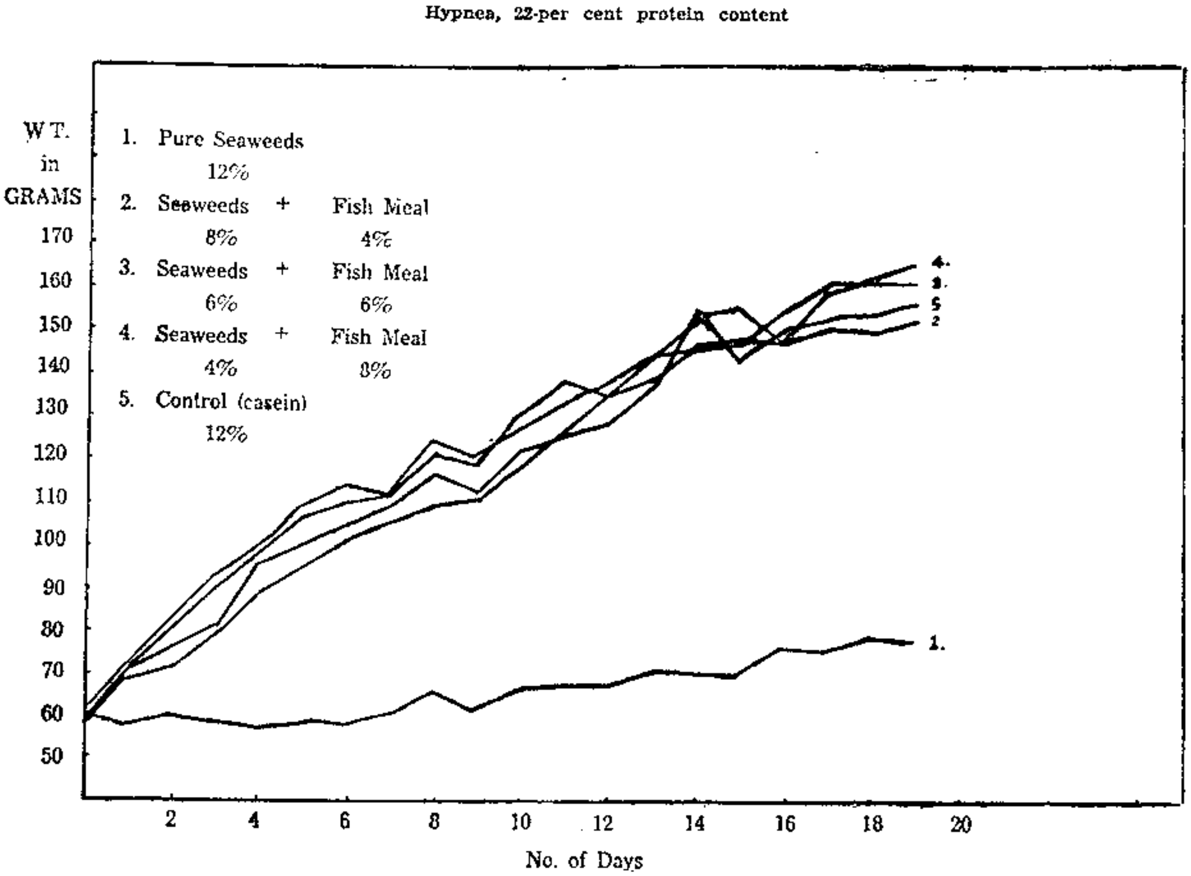

FIG. 1. Comparative rate of growth of different proportions of seaweed in the diet of experimental rats.

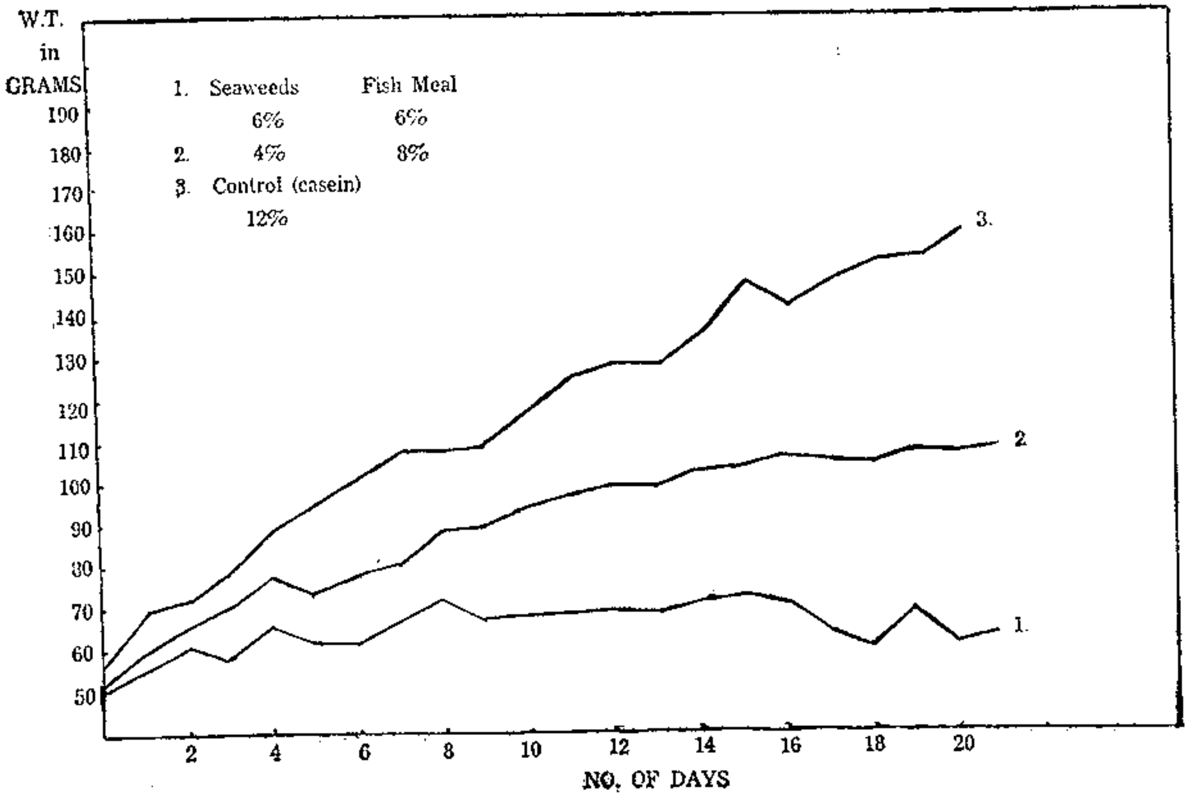
Sargassum, 9.7-per cent protein content

FIG. 2. Comparative rate of growth of different proportions of seaweed in the diet of experimental rats.

Hydroclathus cancellatus, 9.28-per cent protein; Asparagopsis taxiformis, 11.83-per cent protein

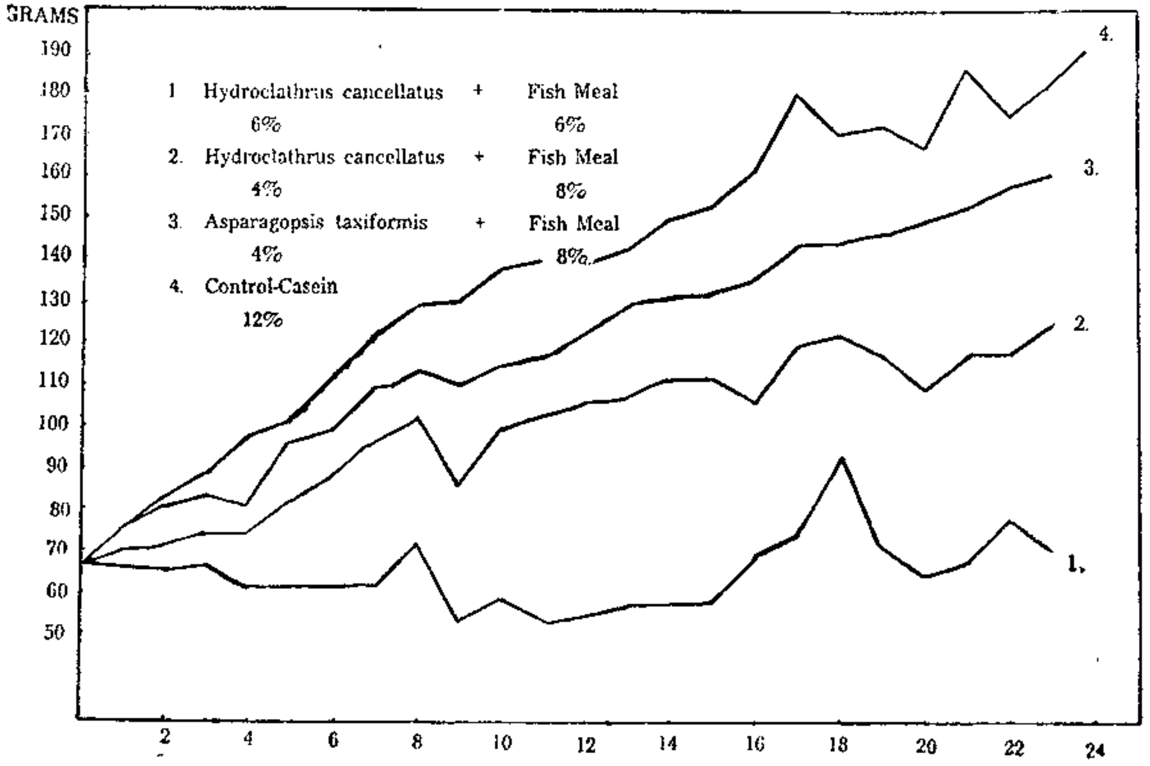

FIG. 3. Comparative rate of growth of different proportions of seaweed in the diet of experimental rats.

It is very interesting to note that these investigations confirm the findings of earlier investigators on the biology of bañgos (*Chanos chanos*), that this fish is essentially planktonic in food habits from the earlier stages up to the adult. Fishpond owners use the *Enteromorpha intestinales* and *Cladophora* with the impression that they provide the bañgos with food for growth. These seaweeds actually provide the planktonic constituents of the water with the ideal environmental conditions necessary for their growth and multiplication like the maintenance of the ideal physico-chemical and biological properties of the water. The bañgos feed on these algæ to a limited extent and it starts when the seaweeds begin to disintegrate. Natural disintegration of these algæ transforms the water into a more favorable medium for normal planktonic growth and becomes a factor in the maintenance of the existing food chain in that body of water.

Hydroclathrus cancellatus which contains 9.28-per cent protein does not seem to support growth at the level of 6-per cent seaweed meal and 6-per cent fish meal. However, at the level of 4-per cent seaweed and 8-per cent fish meal, the effect on the growth of the rats was better although it was not as good as *Asparagopsis*. Further experiments will be made to determine if the stage of development of the thallus body of the seaweeds affect the quality of its protein content.

CONCLUSION

The chemical composition of seaweeds varies according to their habitat and stage of growth at harvest time. In view of this fact, a thorough study of the seasonal variation in their composition caused by the factors mentioned above is essential if the desired components during the period of their optimum concentration are to be obtained.

Harvesting and utilization of seaweeds with low nutrient value would, instead of yielding profit, incur big losses in the feed milling industry.

Seaweeds contain big amounts of indigestible carbohydrates so that they should be used in combination with more nutritive material like fish meal. Their crude fiber content which is high and undigestible, gives bulk to the food residue, facilitating its elimination.

SUMMARY

The utilization of seaweeds for human and animal food has attracted the attention of Philippine industrialists because of their abundance in Philippine waters and their nutritional qualities, digestibility and potentialities for growth. Because of these properties they are being used extensively in Great Britain and the Scandinavian countries as feeds for animals. The major constituent of seaweed is carbohydrate; the minor constituents include protein, fat, minerals and vitamins. Several species of local seaweeds and algæ have been experimented on to determine their potentialities as sources of cheap protein concentrates that could be substituted for fish meal in the preparation of animal feeds. From this project, full development of a stable seaweed industry could be realized for the benefit of the poultry, piggery, and livestock industries.

Results of standard bio-assay experiments using rats as laboratory animals reveal the following: (a) *Asparagopsis taxiformis* (bulaklak bato) indicate that while its protein content is 11.82 per cent, it can support growth at a proportion of 4-per cent seaweed meal to 8-per cent fish meal. Inspite of the high protein content of *Enteromorpha* (bitukang manok), (17.82 per cent) and *Cladophora* (lumut jusi), (19.02 per cent), their effect on the growth of rats were not satisfactory at the levels which were effective for *Asparagopsis.* Combination of equal proportions of *Enteromorpha, Cladophora* and fish meal was tried and the effect was better although it was still below the control group. *Hydroclathrus cancellatus* (Bal-balulang) which contains 9.28-per cent protein can support growth at the level of 4-per cent seaweed and 8-per cent fish meal.

Hypnea, which was incorporated in three proportions, gave the highest rate of growth. *Asparagopsis* and *Sargassum* came second followed by *Hydroclathrus, Cladophora,* and *Enteromorpha.* Chemical composition of seaweeds vary according to the habit, stage of growth and the time of harvest. It is essential to undertake a close study of the seasonal variation on the composition to get the desired components during the period of optimum concentration. Harvesting and utilization of seaweeds with low nutrient value would, instead of yielding profit, incur big losses in the feed milling industry.

ACKNOWLEDGMENT

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TABLE 1.—*Growth studies on Enteromorpha intestinales and gladophora sp.*

Ingredients	*Interomorpha intestinales* Pure seaweed sample 12-per cent protein	*Interomorpha intestinales* 8-per cent protein Fish meal 4-per cent protein	*Cladophora* Pure seaweed sample 12-per cent protein	*Cladophora* 8-per cent Fish meal 4-per cent protein	*Interomorpha* 4-per cent protein *Cladophora* 4-per cent protein Fish meal 4-per cent protein	Casein 12-per cent Control
	gm	*gm*	*gm*	*gm*	*gm*	*gm*
Seaweeds	0.67	0.44	0.63	0.42	0.224	——
					0.210	——
Fish meal	——	0.060	——	0.060	0.060	——
Cornstarch	86.33	86.5	86.37	86.5	86.5	86.9
Lard	3.0	3.0	3.0	3.0	3.0	3.0
Yeast	8.0	8.0	8.0	8.0	8.0	8.0
Salt mixture No. 51	2.0	2.0	2.0	2.0	2.0	2.0
Cod liver oil	4 drops	4 drops	4 drops	4 drops	4 drops	4 drops
Casein						0.144

TABLE 2.—*Growth studies on Hydroclathrus sp. and Asparagopsis taxiformis.*

Ingredients	*Hydroclathrus cancellatus* 6-per cent protein Fish meal 6-per cent protein	*Hydroclathrus cancellatus* 4-per cent protein Fish meal 8-per cent protein	*Asparagopsis taxiformis* 4-per cent protein Fish meal 8-per cent protein	Casein Control
Seaweeds	*gm*	*gm*	*gm*	*gm*
Fish meal	0.646	0.431	0.338	
Cornstarch	0.091	0.121	0.121	
Lard	86.3	86.5	86.0	86.9
Yeast	3.0	3.0	3.0	3.0
Salt mixture No. 51	8.0	8.0	8.0	8.0
Cod liver oil	2.0	2.0	2.0	2.0
Casein	4 drops	4 drops	4 drops	4 drops
				0.144

TABLE 3.—*Growth studies on Hypnea.*

Ingredients	*Hypnea* Pure seaweed sample 12-per cent protein	*Hypnea* 8-per cent protein Fish meal 4-per cent protein	*Hypnea* 6-per cent protein Fish meal 6-per cent protein	*Hypnea* 4-per cent protein Fish meal 8-per cent protein	Casein 12-per cent protein Control
	gm	*gm*	*gm*	*gm*	*gm*
Seaweeds	0.542	0.361	0.271	0.180	——
Fish meal	——	0.060	0.091	0.121	——
Cornstarch	86.5	86.6	86.6	86.7	86.9
Lard	3.0	3.0	3.0	3.0	3.0
Yeast	8.0	8.0	8.0	8.0	8.0
Salt mixture No. 51	2.0	2.0	2.0	2.0	2.0
Cod liver oil	4 drops	4 drops	4 drops	4 drops	4 drops
Casein	——	——	——		0.144

TABLE 4.—*Growth studies on Sargassum.*

Ingredients	Seaweeds 6-per cent protein Fish meal 6-per cent protein	Seaweeds 4-per cent protein Fish meal 8-per cent protein	Casein 12-per cent protein
	gm	*gm*	*gm*
Seaweeds	0.618	0.412	—
Fish meal	0.091	0.121	--
Cornstarch	86.3	86.5	86.9
Lard	3.0	3.0	3.0
Yeast	8.0	8.0	8.0
Salt mixture No. 51	2.0	2.0	2.0
Casein	4 drops	4 drops	4 drops
Cod liver oil	—	—	0.144

TABLE 5.—*Proximate chemical composition of seaweeds used in the experiment.*

	Moisture	Ash	Crude fat	Crude fiber	Iodine	Mannitol	Nitrogen	Crude protein	Alginic acid
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
I. *Cladophora*	12.815	50.37	1,493	9,997	8.145	6.671	3.04	19.02	7.585
II. *Asparagopsis taxiformis*	6.8355	5.286	2.747	6.4565	7.00	9.78	1.81	11.83	0.178
III. *Enteromorpha intestinales*	9.607	32.44	3.697	5.56	0.151	7.12	2.85	17.82	0.203
IV. *Hydroclathrus cancellatus*	7.457	48.98	2.859	5.818	0.107	17.42	1.48	9.28	7.26
V. *Hypnea* sp.	12,82	44.7	2.4685	6.300	0.9245	24.48	3.54	22.13	1.549
VI. *Sargassum*	18.055	31.68	1.752	51.48	50.47	20.24	1.55	9.70	6,165

NOTE: The above analyses were performed at the Central Technological Laboratories of the Philippines Fisheries Commission, Dagat-dagatan Experimental Station, Navotas, Rizal.

TABLE 6.—*Mineral composition of seaweeds used in the experiment.*

Seaweeds	Aluminum	Calcium	Iron	Potassium	Magnesium	Sodium	Bromine
	ppm	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
I. *Cladophora*	8.30	8.65	9.00	1.92	2.35	3.80	1.30
II. *Asparagopsis taxiformis*	9.00	8.50	2.20	14.00	6.60	2.10	0.42
III. *Enteromorpha intestinales*	7.10	9.50	2.00	19.00	6.70	2.02	0.90
IV. *Hydroclathrus cancellatus*	9.00	12.40	19.60	1.94	3.00	0	0.40
V. *Hypnea* sp.							
VI. *Sargassum*	6.85	19.50	16.30	2.13	2.04	1.46	3.15

NOTE: The above analyses were performed at the Araneta University in connection with the NSDB-assisted project on Philippine seaweeds.

TABLE 7.—*Gain in weight, food and protein intake of rats during the six weeks study.*

Seaweeds	Rat No.	Weight gain	Food intake	Protein intake
		gm	*gm*	*gm*
Hypnea	1	7.2	262.2	30.50
12-per cent protein	2	6.0	238.8	28.66
	3	5.6	252.5	30.30
	4	4.8	209.5	25.14
	5	0.5	235.6	28.27
	6	3.3	331.5	27.78
	7	7.2	313.6	37.63
	8	8.0	231.3	27.76
Average	....	5.3	246.9	29.50
Hypnea	1	39.1	334.2	40.11
8-per cent protein	2	70.2	422.9	50.75
	3	47.3	385.0	46.20
Fish meal	4	63.0	432.7	51.92
4-per cent protein	5	49.9	402.9	48.35
	6	60.1	416.3	49.96
	7	57.2	399.4	46.73
	8	65.1	418.1	50.17
Average	....	56.5	401.4	48.09
Hypnea	1	63.4	408.5	49.02
6-per cent protein	2	78.1	393.3	47.20
	3	47.3	365.5	43.86
Fish meal	4	51.8	427.6	51.31
6-per cent protein	5	65.9	396.1	47.53
	6	54.3	397.1	47.65
	7	62.1	364.4	43.73
	8	56.5	389.0	46.68
Average	....	59.9	392.7	47.12

TABLE 8.—*Gain in weight, food and protein intake of rats during the six weeks study.*

	Rat No.	Weight gain	Food intake	Protein intake
		gm	*gm*	*gm*
Hypnea	1	63.3	400.7	48.08
4-per cent protein	2	64.2	399.0	47.88
	3	66.3	389.1	46.92
Fish meal	4	62.4	387.5	48.50
8-per cent protein	5	69.9	397.8	47.74
	6	64.9	413.5	49.62
	7	39.3	312.0	34.44
	8	64.0	420.9	52.51
Average	....	61.8	390.1	46.96
Sargassum	1	11.2	326.2	39.14
6-per cent protein	2	2.1	356.2	42.74
	3	19.7	357.9	42.95
Fish meal	4	16.4	361.8	43.42
6-per cent protein	5	22.5	360.3	43.30
	6	20.5	333.7	40.04
Average	....	15.4	349.3	41.93
Sargassum	1	28.5	320.3	38.44
4-per cent protein	2	47.7	320.7	38.48
	3	48.2	326.7	39.20
Fish meal	4	34.0	330.1	39.61
8-per cent protein	5	39.7	314.5	37.74
	6	30.4	347.4	41.69
Average	....	15.4	326.6	39.19
Control	1	77.6	422.4	50.67
Casein	2	37.1	300.1	36.01
12-per cent protein	3	85.0	458.0	55.07
	4	56.9	321.9	38.63
	5	51.9	346.0	41.52
	6	37.7	396.3	47.56
	7	46.2	347.9	41.75
	8	51.5	400.1	48.01
Average	....	55.5	374.1	44.90

TABLE 9.—*Gain in weight, food and protein intake of rats during the six weeks study.*

Seaweeds	Rat. No.	Weight gain	Food intake	Protein intake
		gm	*gm*	*gm*
Hydroclathrus cancellatus	1	25.4	422.0	50.64
4-per cent protein	2	30.4	463.0	55.64
	3	45.8	487.8	58.54
Fish meal	4	34.24	452.2	54.26
8-per cent protein	5	26.9	422.7	50.72
	6	24.9	450.3	54.04
	7	29.96	438.0	52.56
	8		425.3	51.04
Average	....	30.8	445.2	53.43
Asparagopsis taxiformis	1	39.2	351.2	42.14
4-per cent protein	2	30.0	347.5	41.60
	3	55.0	388.7	46.64
Fish meal	4	47.89	379.7	45.56
8-per cent protein	5	44.7	368.1	44.17
	6	40.8	367.3	44.08
	7	47.86	388.3	46.60
	8	35.16	347.7	41.72
Average	....	46.3	367.3	44.06
Control	1	71.4	424.9	50.99
Casein	2	36.8	400.0	48.00
12-per cent protein	3	81.61	416.6	49.99
	4	65.0	412.7	45.53
	5	86.9	404.8	48.58
	6	61.9	418.8	50.26
	7	67.2	420.2	50.43
	8	58.4	408.5	49.02
Average	....	66.1	413.3	49.10

THE INCIDENCE OF ASCARIS OVA IN VEGETABLES COMMONLY EATEN RAW IN THE PHILIPPINES

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TWO PLATES

The vegetables, whose succulent parts are sometimes eaten raw by many people in the Philippines, like many other cultivated plants, are produced chiefly by growing in the soil, the storage bin of plant nutrients. The practical significance and productivity of any soil is determined partly by the abundance and kinds of living organisms — green plants, small multicellular animals and microorganisms associated with it, as it has often been said, "no soil without life, no life without soil." Unfortunately, among the forms of small multicellular animals occurring now and then in the soil are the parasitic worms which exist commonly as eggs and larval forms or cysts. This is especially true in vegetable gardens which are continually exposed to pollution with animal dung or human excrements. One of them is an ascarid (*Ascaris lumbricoides* L.), a roundworm which is parasitic in the human intestine. Its prolificness is astounding, being capable, according to Cram,(2) of producing 27,000,000 eggs per mature female, so that one gram of feces discharged from a person infected with ascariasis, may contain more than 2,000 eggs.

Owing to their close and grappling contact with the soil, the vegetables, particularly those raised subconsciously in polluted gardens certainly serve as agents for the spread of *A. lumbricoides* ova and effective transfer of this parasite into the human intestine by eating them in a raw state.

PREVIOUS WORKS ON THE INTESTINAL ROUNDWORM (A. LUMBRICOIDES L.) IN THE PHILIPPINES

In a summarized report on parasitic worms prevalent in the Philippines, Tubangui(9) listed 336 helminths, 138 of which were nematodes, and pinpointed ascariasis caused by *A. lumbri-*

coides as the predominating type of parasitic infestation. In their appraisal of the prevalence of intestinal helminths in adult Filipinos, Schwartz and Tubangui(8) centered their focus to *A. lumbricoides* as the preponderant parasite, with infestations averaging 54.14 per cent based on examinations of 59,154 Filipinos made by several investigators during a period extending from 1908 to 1917. Pesigan(7) observed an incidence of 85.77 per cent for ascariasis in nearly all the provinces in Mindanao, Leyte, Samar, Bohol, Sorsogon, and Mindoro. Of the 85 school-aged children that she examined in Oriental Mindoro, Velasquez (10) found 91.77 per cent to be positive for ascariasis. From the same study, she also reported on the role played by various farm animals (chickens, horses, goats, cats, and dogs) as well as common insects (ants, flies, coackroaches, etc) in the dissemination of Ascaris ova. In a survey of various age classes and professional groups for intestinal parasites in Olongapo, Zambales Province, Kuntz(5) found an infestation of 23.81 per cent for ascariasis.

The above facts not only show the predominance of ascariasis over all parasitic worm infestations in Philippine population but also reveal its strikingly discrepant incidence in urban and rural areas and also in different age classes and occupational groups. In this connection, Velasquez(10) observed that despite rigid control measures and modern ways of therapy, ascariasis still predominates in rural areas with incidence usually greater in the lower-income than in the higher-income groups. This observation was fully substantiated by Kuntz.(5)

Only recently, in his report on the occurrence of intestinal parasites on raw vegetables, Paguio(6) mentioned the presence of cysts, ova and larvæ of different intestinal worms (*A. lumbricoides, Trichuris trichiura, Enterobius vermicularis* and *Trichostrongylus* sp.) and protozoa (*Entamœba*[1] *histolytica, Entamœba coli, Endolimax nana,* and *Giardia lamblia*) on lettuce, "kinchay," American celery, cabbage, onion tops, tomatoes, leeks, cucumber, raddish, onion bulbs, strawberries, and watercress.

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[1] The generic name *Entamœba* as used by Paguio(6) is considered by the authors as synonymous with *Endamœba*, the generic name adopted by most authors in referring to a protozoan species similar to that mentioned above.

OBJECTIVES

To determine the seasonal occurrence of *A. lumbricoides* ova on vegetables gathered from gardens or sold in markets, "talipapa" [2] and groceries in Manila and Baguio City and oftentimes eaten raw by a large portion of the population and to evaluate the effectiveness of ordinary kitchen method of washing in ridding them of the worm contaminants.

MATERIALS AND METHODS

Collection of vegetables.—The 16 different kinds of vegetables used in this study were from the dry- and wet-season[3] crops in 1961 and 1962. They were either collected directly from the gardens in the lowland city of Manila and suburbs, and in the mountain city of Baguio, Benguet Province or purchased from the groceries and stalls in public markets and "talipapa." They were wrapped separately in clean paper and brought to the Biological Research Center Laboratory, National Institute of Science and Technology, Manila for detailed observation for the presence of the parasitic roundworm (*A. lumbricoides*) ova.

Sampling and preparation of materials for microscopic examinations.—In sampling the vegetable materials, proper sanitary measures were observed to prevent contamination of the samples with *A. lumbricoides* ova from extraneous sources or from laboratory handlers. The inedible portions of vegetables were removed. The edible portions were clipped or cut off randomly and then sampled into 20-gram lots. The lot termed "washed sample" elsewhere in this paper was then 20-gram lot which had been washed in three changes of tap water in a clean basin, a procedure ordinarily followed by housewives in cleaning vegetables to be eaten raw at their tables. When there was no more water dripping, the washed material was flooded with 100-ml distilled water to dislodge any egg, cyst or larvæ of *A. lumbricoides* which might have still adhered superficially to it. In the other 20-gram lot termed "unwashed sample," no prewashing with tap water was performed before flooding it with 100 ml distilled water to dislodge from it the parasite. Distilled-water washings from washed and unwashed lots were collected or set aside for use in examinations for the presence of *A. lumbricoides* ova. In preparing samples for examination for the presence of the ova from dry- and wet-season crops, the procedure for the preparation of unwashed sample was followed.

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[2] "Talipapa" is a local term for an unauthorized, open market catering to low-income groups of people.

[3] The "dry season" considered in this report is the sunny weather or period of scant rain usually extending from the second half of December to the first half of May; "wet season" is the rainy period spanning over the months from the second half of May to the first half of December.

Method of recognizing A. lumbricoides ova.—To facilitate collection of *A. lumbricoides* ova from distilled-water washings from both washed and unwashed lots, the zinc-sulfate-floatation-technique (ZSFT) described by Faust, et al.(3) was followed. This treatment caused the ova to rise to and float on the surface of the distilled-water washings. The surface films of both the washed and unwashed samples were examined microscopically for the presence of *A. lumbricoides* ova which can be recognized by their characteristic features described as follows:

A. lumbricoides eggs are yellowish or brownish and can easily be mistaken for soil particles if one is unfamiliar with their characteristic features. They are oval to ovoid in shape and vary in size from 60 to 70 microns long by 40 to 50 microns wide (Plate 1, figs. 1–2). The fertilized eggs can be recognized by the presence of a warty albuminous layer enveloping the shell which encloses the granular cytoplasmic contents (Plate 2, figs. 1–2). The unfertilized eggs, on the other hand, are a little more elongate in shape (Plate 1, fig. 3), and bear amorphous contents.

RESULTS AND DISCUSSION

The result of the microscopic examinations of sixteen vegetables for contamination with *A. lumbricoides* ova showed positively the presence of the latter on all of them. This indicates that these vegetables can be agents for the effective transmission of the parasitic worm under investigation and can certainly be a factor for the increased prevalence of ascariasis in several regions in the Philippines, which in certain cases, reach epidemic proportions, particularly in rural communities where hygienic and sanitary way of living is not generally given much attention. The absence of the ova on "ampalaya" (*Momordica charantia* L.), carrot (*Daucus carota* L.), spinach (*Spinacia oleracea* L.) and "uansoi" (*Coriander sativum* L.) shown in Table 1, is presumed to be due either to their nonexistence in an unpolluted garden in which the vegetables were grown or to their dislodgement when washed with water before selling them in the market, "talipapa" or grocery.

The presence of *A. lumbricoides* ova on both dry- and wet-season crops of vegetables (Table 1) insinuates that at any time of the year, the latter may serve as disseminators of ascariasis if eaten raw as salads or used as garnish. The slightly higher incident of *A. lumbricoides* ova on wet-season than on dry-season vegetables could be due to the more frequent occurrence and greater abundance of rains which form surface floods that spread feces-contaminated soils throughout the garden. When the rains cease and the floods subside, *A. lumbricoides* which may occur in different stages (larva, egg, or cyst), mostly eggs, is left on the soil surface and can remain alive or viable for a reasonably long time, particularly in soils which are retentive of of moisture such as the loamy type seen in most gardens visited in Manila and Baguio City. Splashes from new rains and from water sprinkled during watering of vegetables, transfer polluted particles of soil to the plants, thereby, contaminating them also.

It will also be seen in Table 1 that, generally, the samples from Baguio City show higher percentage of contamination with *A. lumbricoides* ova than those from Manila. This is due, presumably, to the cooler climate of the mountain city of Baguio which is responsible for delaying the desiccation of the eggs, thus, lengthening the period of their viability and to the more extensive use, in this place, of animal manure and perhaps night soil, which many people talk of casually as organic fertilizer, for enriching the gardens in essential plant nutrients.

Tables 1 and 2 show that there is no species of vegetable which is free from contamination with *A. lumbricoides* ova. Celery (*Apium graveolens* L.), lettuce (*Lactuca sativa* L.), mustard (*Brassica integrifolia* West O. E. Schulz.), leek (*Allium esporrum* L.), onion (*Allium cepa* L.), tomato (*Lycopersicum esculentum* L.) and strawberry (*Fragaria vesca* L.) showed, on the average, higher percentage of contamination than the other vegetables examined. A more critical study of their external structures revealed that they generally possess surfaces roughened by many small ridges and furrows and by hairy coverings, by means of which they are capable of securely lodging the ova of *A. lumbricoides*. In the minute grooves and wrinkles, the ova are well sheltered from the desiccative action of the sun. It is also observed that the vegetables showing higher percentage of contamination are those that grow close to the ground like lettuce, parsley, leek, tomatoes, onion, etc. The tall and climbing vegetables like spinach and ampalaya, whose edible portions are picked usually at heights not easily reached by splashes from the ground, are less contaminated with ascarid ova. Water-cress (*Roripa nasturtium aquaticum* Hayek), grown in fresh water in the mountain city of Baguio was found to be heavily contaminated with *A. lumbricoides* ova. It was observed during the collection of the samples that the fresh-water streams in which they were grown was polluted with sewage coming from one of the sewers in the upper nearby community.

Contamination can also be traced to the water used by most of the vendors to freshen the vegetables for sale to the public. Such water might be contaminated with *A. lumbricoides* eggs from handlers themselves. Moreover, in most stalls, especially in the markets and "talipapas" in Manila, the vegetables are mixed with other items such as fish, meat and fruits on which flies (*Musca* sp., *Calliphora* sp., and *Lucilia* sp.), commonly found breeding in fecal matter, feed. Any ascaris ova that might have been transferred by the flies on these goods could contaminate the vegetables. Furthermore, in the same talipapas, the vegetables, like other items for sale, are frequently touched by so many hands which may have carried the ascaris ova.

Among the individual vegetables examined (Table 1), strawberry, lettuce and celery show the highest percentage of contamination on both dry- and wet-season crops. This is in congruity with the findings of Paguio.(6)

Table 2 indicates that the ordinary way of washing vegetables as practiced in most Filipino homes does not seem to be sufficiently thorough as to rid the vegetables of *A. lumbricoides* ova. Such method would even be less efficacious if the vegetables bear rough surfaces such as on those discussed ahead.

The ineffectiveness of the ordinary way of washing to rid fresh vegetables of *A. lumbricoides* ova, shown in Table 2 serves as a frightful warning to eaters of raw vegetables and to those in charge of the cuisine to observe all necessary precautions for ridding the herbage of parasitic worm contaminants before eating them.

Some measures for dislodging ascaris ova have been suggested by Velasquez(10) who showed that the cleansing and germicidal properties of either soap or lye is beneficial at 70° to 100°C. The same author reports that momentary dippings in water at 70°C or higher kill the *A. lumbricoides* ova. The present authors, however, observed that this temperature tends to scorch the vegetables, thereby, losing their crispness and tenderness. In her other suggestion, Velasquez(10) points to the effectiveness of 6 to 7 washings of the vegetables in running water in dislodging the *A. lumbricoides* eggs from the vegetables.

It is recommended that if night soil is to be used as organic fertilizer for vegetables, it should be handled properly and should be so treated as to render it free of any viable stage of *A. lumbricoides.* In this connection, Kobayashi and Komiya(4) observed that, by placing night soil in storage tanks provided with covers during dry weather and allowing it to undergo fermentation, together with a certain amount of compost, the eggs of *A. lumbricoides* lose their viability in 3 to 4 weeks due to the high temperature produced during fermentation. They emphasized that this procedure requires strict sanitary measures to prevent dissemination of all forms of parasites and pathogenic microorganisms which might be present in the fecal matter.

SUMMARY

1. Vegetables gathered from Baguio City and Manila and sold in markets, groceries, and talipapas are found contaminated with *A. lumbricoides* ova at anytime of the year. The smooth-surfaced and climbing types of vegetables are less contaminated compared with the rough-surfaced, low- or non-climbing types. Vegetables grown in the mountain city of Baguio show higher percentage of contamination than those grown in lowland city of Manila.

2. The ordinary manner of washing vegetables in most Filipino homes is not sufficient to dislodge completely the ova of *A. lumbricoides* from the vegetables commonly eaten raw by Filipinos. Six to seven washings of vegetables in running water have been suggested as an effective way of freeing them of the parasitic worm contaminants.

3. Proper disposal of waste and sewage material as well as proper handling and treatment of night soil should be strictly observed in areas close to vegetable gardens, in order to prevent the spread of the parasites and other pathogenic microbes.

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TABLE 1.—*Dry- and wet-season vegetables examined and the percentage of samples found contaminated with A. lumbricoides ova.*

Vegetable[1]	Portions examined	Origin of sample		Dry season crop			Wet season crop		
		Place	Source	No. of samples examined	No. of ascarid positive samples	Ascarid contamination	No. of samples examined	No. of ascarid positive samples	Ascarid contamination
						Per cent			*Per cent*
Alugbati (*Basella rubra* L.)	Leaves & tender stems	Manila	Market	48	0	0.0	28	2	7.14
Ampalaya (*Momordica charantia* L.)	Leaves & tender stems	Manila	Market	96	0	0.0	56	0	0.0
Cabbage (*Brassica oleracea* L. var. *capitata* L.)	Leaves	Manila	Garden	10	0	0.0	12	0	0.0
			Market	18	0	0.0	15	1	6.66
Do	do	Baguio	Garden	18	0	0.0	20	0	0.0
			Market	25	1	4.0	30	0	0.0
Carrot (*Daucus D. carota* L.)	Storage root	Manila	Garden	3	0	0.0	4	0	0.0
			Market	15	0	0.0	15	0	0.0
Do	do	Baguio	Garden	20	0	0.0	24	0	0.0
			Market	16	0	0.0	20	0	0.0

[1] The common and scientific names of vegetables listed in the tables are those adopted by Dr. William Brown (1) in his book "Useful Plants of the Philippines."

TABLE 1.—*Dry- and wet-season vegetables examined and the percentage of samples found contaminated with A. lumbricoides ova*—Continued.

Vegetable [1]	Portions examined	Origin of samples		Dry season crop			Wet season crop		
		Place	Source	No. of samples examined	No. of ascarid positive samples	Ascarid contamination	No. of sample examined	No. of ascarid positive samples	Ascarid contamination
Celery (*Apium graveolens* L.)	Leaves & tender stems	Manila	Garden	11	6	5.45	11	8	7.27
			Market	18	14	77.77	18	16	88.88
Do	do	Baguio	Market	10	6	66.00	14	2	14.28
			Grocery	1	0	0.0	3	2	66.66
			"Talipapa"	4	2	50.00	4	2	50.00
Leek (*Allium porrum* L.)	Leaves & tender stems	Manila	Garden	18	1	5.55	22	2	9.18
			Market	17	1	5.88	59	3	5.08
Do	do	Baguio	"Talipapa"	13	0	0.0	8	1	12.50
			Garden	0	0	0.0	39	0	0.0
		Manila	Garden	32	8	25.00	21	9	42.85
			Market	10	3	30.00	28	7	25.00
Lettuce (*Lactuca sativa* L.)	Leaves	Baguio	Garden	21	10	47.61	28	18	64.28
			Market	9	1	11.11	8	4	50.00
Do	do	Manila	Garden	71	2	2.81	51	4	7.84
			Market	54	1	1.85	31	1	3.22
			"Talipapa"	30	0	0.0	20	1	5.00
Mustard (*Brassica integrifolia* West O.E. Schulz)	Leaves	Baguio	Market	44	1	2.27	50	5	10.00
			Garden	25	0	0.0	20	1	5.00
Onion (*Allium cepa* L.)	Leaves & bulb	Manila	Garden	104	4	3.84	52	5	9.61
			Market	74	2	2.70	50	2	4.00
Do	do	Baguio	Garden	52	3	5.76	50	3	6.00
			Market	50	3	6.00	36	6	16.66

Parsley (*Petroselinum hortense* Hoffm.)	Leaves	Manila	Grocery	5	0	0.0	3	0	0.0
Do	do	Baguio	Grocery	8	0	0.0	12	1	8.33
			Market	3	1	33.3	9	0	0.0
Raddish (*Raphanus sativus* L.)	Young leaves & tender stems	Manila	"Talipapa"	12	0	0.0	13	0	0.0
			Market	10	0	0.0	27	2	7.40
Do	do	Baguio	Market	8	0	0.0	5	0	0.0
			Grocery	10	0	0.0	3	0	0.0
Spinach (*Spinacia oleracea* L.)	Young leaves & tender stems	Manila	Garden	28	0	0.0	26	0	0.0
			Grocery	4	0	0.0	0	0	0.0
Do	do	Baguio	Grocery	2	0	0.0	2	0	0.0
Strawberry (*Fragaria vesca* L.)	Fruits	Baguio	Garden	12	11	91.66	16	12	75.00
Do	do								
Tomato (*Lycopersicum esculentum* L.)		Manila	Garden	4	1	25.00	7	0	0.0
			Grocery	2	0	0.0	8	0	0.0
			Market	40	6	15.00	10	2	20.00
			"Talipapa"	25	3	12.00	15	3	0.0
Do	do	Baguio	Grocery	18	3	16.66	10	0	0.0
			Garden	9	3	33.00	20	5	25.00
			Market	2	0	0.0	8	0	0.0
Unsol (*Coriander sativum* L.)	Leaves & tender stems	Manila	Garden	30	0	0.0	0	0	0.0
			Market	17	0	0.0	12	0	0.0
			"Talipapa"	4	0	0.0	2	0	0.0
Do	do	Baguio	Market	0	0	0.0	6	0	0.0
Watercress (*Roripa nasturtium aquaticum* Hayek)	Leaves & tender stems	Baguio	Garden	6	4	66.66	40	9	22.50
			Market	2	0	0.0	12	2	16.66

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[1] The common and scientific names of vegetables listed in the table are those adopted by Dr. William H. Brown(1) in his book "Useful Plants of the Philippines."

TABLE 2.—*Percentage of incident of A. lumbricoides ova on "washed" and "unwashed" samples of vegetables.*

Vegetable	No. of samples examined	No. of positive samples	Contamination	No. of samples examined	No. of positive samples	Contamination
			Per cent			*Per cent*
Alugbati (*B. rubra* L.)	20	1	5.0	20	—	—
Ampalaya (*M. charantia*)	38	1	2.6	38	1	2.6
Cabbage (*B. oleracea* L.) var. *capitata* L.)	60	1	1.7	60	1	1.7
Carrot (*D. carota* L.)	42	3	7.1	42	1	2.3
Celery (*A. graveolens* L.)	47	26	55.3	47	20	42.5
Leek (*A. porrum* L.)	88	5	5.7	88	2	2.3
Lettuce (*L. sativa* L.)	228	5	2.2	228	4	1.8
Mustard (*B. integrifolia* O. E. Schulz)	55	4	7.2	55	2	3.6
Onion (*A. cepa* L.)	234	18	7.7	234	10	4.3
Parsley (*P. hortense* Haffm.)	44	—	—	44	—	—
Raddish (*R. sativus* L.)	24	3	12.7	24	1	4.1
Spinach (*S. oleracea* L.)	31	1	3.2	31	—	—
Strawberry (*F. vesca* L.)	14	13	92.9	14	10	71.4
Tomato (*L. esculentum* L.)	88	15	17.0	88	10	11.3
"Uansoi" (*C. sativum* L.)	58	1	1.7	58	—	—
Watercress (*R. nasturtius aquaticum* Hayek)	38	2	5.3	38	1	2.6

ILLUSTRATIONS

[Photomicrographs by Mr. Nemesio Bercasio, Technical Photographer, Division of Documentation, National Institute of Science and Technology, Manila.]

PLATE 1

Ova of *Ascaris lumbricoides* L., which were recovered from vegetable washings, in different stages of development.

FIG. 1. Early cleavage, *a—b;* morula stage, *c;* empty and ruptured egg shells after larvæ have been released, *d;* about × 300.

2. Immature egg, *a;* unfertilized ovum, *b;* fertilized ova, *c*. Note the variation in size and shape of the ova; about × 300.

3. Unfertilized ovum showing early, *a;* and late, *b;* cleavage stage; about × 300.

PLATE 2

1. Fertilized ovum showing the characteristic warty structure on the shell; about × 300.

2. Fertilized ovum containing a fully developed embryo; about × 300.

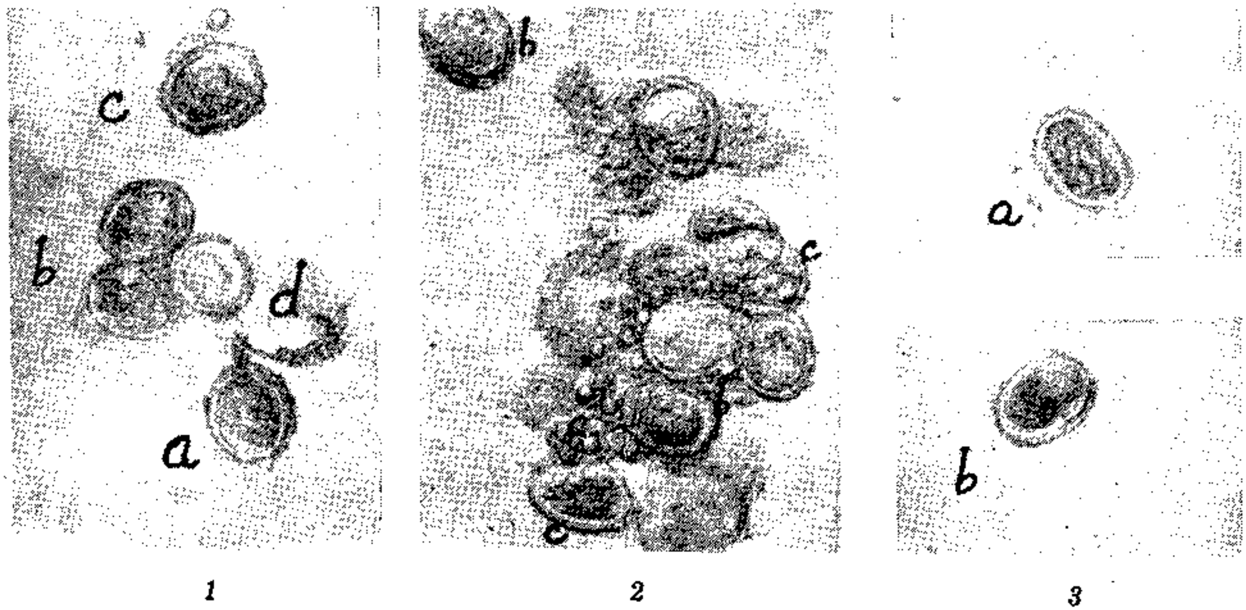
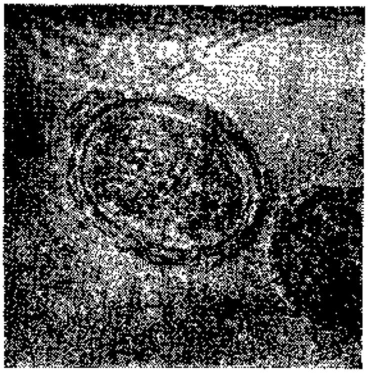


PLATE 1.



1

2

PLATE 2.

PUBLICATIONS AVAILABLE

CHECKLIST OF THE ANTS (HYMENOPTERA: FORMICIDAE) OF ASIA. By J. W. Chapman and S. R. Capco. Institute of Science and Technology Monograph 1 (1951) new series. Paper, 372 pages. Price, $2.00, United States currency.

NOTES ON PHILIPPINE MOSQUITOES, XVI. GENUS TRIPTEROIDES. By F. E. Baisas and Adela Ubaldo-Pagayon. Institute of Science and Technology Monograph 2 (1952) new series. Paper, 198 pages with 23 plates and 4 text figures. Price $2.50, United States currency.

A REVISION OF THE INDO-MALAYAN FRESH-WATER FISH GENUS RASBORA. By Martin R. Brittan. Institute of Science and Technology Monograph 3 (1953) new series. Paper, 224 pages with 3 plates and 52 text figures. Price, $2.50, United States currency.

SECURING AQUATIC PRODUCTS IN SIATON MUNICIPALITY, NEGROS ORIENTAL PROVINCE, PHILIPPINES. By Donn V. Hart. Institute of Science and Technology Monograph 4 (1956) new series. Paper 84 pages with 22 text figures and 8 plates. (Price, $1,25, United States currency.

AN ECOLOGICAL STUDY OF THE KOUPREY, NOVIBUS SAUVELI (URBAIN). By Charles H. Wharton. Institute of Science and Technology Monograph 5 (1957) new series. Paper, 111 pages with 11 plates and 16 text figures. Price, $1.25, United States currency.

FERN FLORA OF THE PHILIPPINES. By Edwin B. Copeland. Institute of Science and Technology Monograph 6, Vols. 1—3 (1958—1960) new series. Vol. 1, 191 p., Paper, Price, $1.25; Vol. 2, 193—376 p., Paper, Price $1.75; Vol. 3, 377—577 p., Paper, Price, $1.75, United States currency.

THE PHILIPPINE PIMPLINI, POEMENIINI, RHYSSINI, AND XORIDINI. By Clare R. Baltazar. National Institute of Science and Technology Monograph 7 (1961) new series. Paper, 120 pages with four plates. Price, $1.50, United States currency.

PACIFIC PLANT AREAS. Edited by C.G.G.J. Van Steenis. National Institute of Science and Technology Monograph 8, Vol. 1 (1963) new series. Paper, 246 pages lith 26 maps. Price, $3.00, United States currency.

INDEX TO THE PHILIPPINE JOURNAL OF SCIENCE, VOL. 59 (1936) TO VOL. 79 (1950). By Angel Y. Lira. National Institute of Science and Technology Monograph 9 (1963) new series. Paper, 325 pages. Price $3.00, United States currency.

THE ARCHAEOLOGY OF CENTRAL PHILIPPINES. By Wilhelm G. Solheim, II. National Institute of Science and Technology Monograph 10 (1964) new series. Paper, 235 pages with 29 text figures and 50 plates. Prices, paper, $3.00; cloth, $6.00, United States currency.

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